Research Institute of Molecular Pathology

VIENNA BIOCENTER

IMP 2002

Boehringer Ingelheim

Contents

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The IMP is a basic research institute within the Boehringer Ingelheim group of companies.

In 2002 the IMP departed for the first time from its traditional career structure and promoted two exceptional Group Leaders, Thomas Jenuwein and Jan-Michael Peters, to Senior Scientists. We congratulate TJ and Jan on their promotions and are delighted that they have decided to continue their excellent research at the IMP.

Our plan to establish a group working on Structural Biology at the IMP came to fruition towards the end of 2002 with the recruitment of Tim Clausen from the Huber lab at the Max Planck Institute in Martinsried. We are confident that Structural Biology will complement the work of our Bioinformatics Department as well as enhance several other scientific projects at the IMP.

We are also delighted to announce the arrival of Ludger Klein as Wittgenstein Group Leader at the IMP, supported by the Wittgenstein Prize of the Austrian Government awarded to Meinrad Busslinger. Ludger comes to the IMP from Harald von Boehmer's lab at Harvard and will build up an independent IMP group working on T cell tolerance.

We said farewell this year to Lukas Huber who moved on from the IMP to a full Professorship and to become Head of the new Institute of Anatomy, Histology and Embryology at the University of Innsbruck. We congratulate Lukas on his new position and wish him all the best. Lukas was a great citizen of the IMP and will be sorely missed, especially as the initiator and organiser of the "Gmunden Ideas Meetings" where IMP scientists got together to exchange ideas with colleagues from Boehringer Ingelheim. This tradition will naturally be continued.

Meanwhile, the preparations for IMBA (Institute of Molecular Biotechnology) adjacent to the IMP continue. Josef Penninger, formerly of Amgen Toronto, signed up as Director of the new institute and was successful in recruiting Barry Dickson from the IMP as his first senior faculty member. This is a great start for IMBA and bodes well for its future.

We are deeply grateful, as ever, to Boehringer Ingelheim for its continued generous support of the IMP. BI stands out in the pharmaceutical world as one of very few companies with such an enlightened attitude to basic research and this has provided the environment for the IMP's success over the past 15 years. I am confident that the IMP will maintain and possibly even surpass its present level of excellence!

Kim Nasmyth

December 2002

IMP by night

THE IMP AND THE CAMPUS VIENNA BIOCENTER

The Research Institute of Molecular Pathology (IMP) is a basic biomedical research center in Vienna, Austria. The research topics currently investigated at the IMP include cell cycle progression, cell differentiation, genetics, oncogenesis, chromosome biology, neuron guidance and stem cell biology. The IMP's major sponsor is Boehringer Ingelheim, an international pharmaceutical company with headquarters in Germany. The knowledge created at the IMP is at the disposal of Boehringer Ingelheim for the development of novel diagnostic and therapeutic concepts.

The IMP opened its doors in 1988. Since then, it has become a major player in the biological sciences, added a new dimension to Austria's research scene and changed its entire neighborhood by attracting more research-driven institutions to the premises. Located next to the IMP are four institutes of the University of Vienna's Faculties of Medicine and Natural Sciences. The IMP and the University maintain close scientific contacts and organize a joint International PhD Program. A "Fachhochschule", a novel type of institution for higher public education, offers curricula in Bioengineering and Biotechnology. Also next door are seven small and middle-sized biotech-companies. Together with the IMP, these institutions form the "Campus Vienna Biocenter".

Three further additions to the Campus are under construction: The Gregor Mendel Institute (GMI), the Institute of Molecular Biotechnology (IMBA) and a commercial building that will rent out lab and office space to start-up companies. The GMI and IMBA will be supported by the Austrian Academy of Sciences. IMBA, under directorship of Josef Penninger, will start operating in 2003. The institute will have close ties to the IMP and share many of its facilities. It will also participate in the Vienna Biocenter International PhD Program and will initially share the Scientific Advisory Board with the IMP.

Currently, more than 900 people from 40 different nations work at the Campus Vienna Biocenter. Among these, approximately 200 are employed at the IMP. All Campus Vienna Biocenter members enjoy a scientifically and socially stimulating environment and take advantage of shared facilities such as the Max Perutz Library at the IMP. A number of events, including seminars and lecture series, are open to all.

"Virtual IMBA" by the architect Boris Podrecca

View of the “Stephansdom” from the Belvedere garden

Belvedere

VIENNA / AUSTRIA

Vienna is the capital city of Austria and home to about 1.5 million people. It is the administrative, political and cultural centre of the country. Geographically, Vienna’s location reflects its historic role at the heart of the large Habsburg empire. Today, with Eastern European countries about to join the European Union, Vienna recognises its chance to become an economic and cultural hub for Central Europe.

Located between the eastern slopes of the Alps and the fertile plains of the river Danube, Vienna enjoys astounding diversity in scenery and activities. One-hour drive can take you to alpine country with opportunities for hiking, climbing or skiing. Travelling in a different direction, you will find yourself at the shores of the lake Neusiedlersee with excellent conditions for sailing and windsurfing in summer and ice-skating in winter. Change your route again and you’ll reach the serene “Waldviertel”, a densely forested, sparsely populated region with ponds, creeks and moors to explore.

Partly within the city’s boundaries is the “Donauauen” National Park, a floodplain forest that extends all the way to the Slovakian border and features rare populations of deer, heron and beaver. Also among Vienna’s unique curiosities: it is the only capital city with a substantial wine production on its grounds. Rolling hills rich in vineyards add to the beauty of the scenery surrounding the city.

If you are interested in culture, art and history, Vienna has more to offer than you can possibly manage to see and do. Opera, concerts and theatre, although performed by world-famous ensembles, are not seen as elitist entertainment but are affordable enough to be enjoyed by everyone.

Art, music and literature are certainly Vienna’s major “obsessions” but there is also a long-standing tradition in science. Founded in 1365, the University of Vienna is the oldest university in the German speaking world and by far the largest one in Austria, offering 130 types of degree programs. In total, there are eight universities in Vienna alone. With a student population of more than 100 000, Vienna offers not only the academic but also the cultural and social infrastructure that comes with student life. So, if you plan to settle down here for a few years, be prepared for numerous visits by friends and relatives.

Michaelertor, Hofburg

YOUR CAREER AT THE IMP

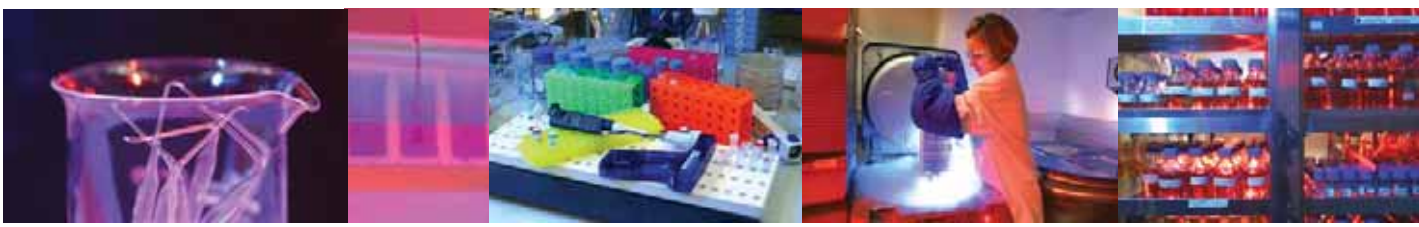
The IMP offers exciting positions at all levels of your research training and career. If you consider joining the IMP, you will find first class research and excellent scientific services. At our institute, you'll be part of a young, international team that uses English as a working language. You will experience a stimulating and focussed atmosphere where science always comes first but social activities are not neglected.

Graduate students join the IMP through the Vienna Biocenter International PhD Program. The doctoral degree is awarded by the University of Vienna. Selection of the students takes place twice a year; PhD contracts typically last 3-4 years. The IMP research groups are well funded to support a number of pre- and postdoctoral positions. Apart from in-house fellowships, IMP scientists are very successful in securing external funding. At present, 35 postdocs from 13 different nations work at the IMP. A substantial travel budget allows scientists to take part in meetings, conferences and courses. The IMP organizes a large international conference every other year and smaller workshops and symposia in-between. An intensive seminar program brings internationally renowned scientists to the IMP at least once a week.

If you come to work at the IMP, you'll obviously come for the science in the first place. We do, however, appreciate your private needs and try to make relocation as smooth as possible. For newcomers, there are several apartments in-house to bridge the time until you have found your own place. When looking for a flat, a staff member will help you negotiate, deal with brokers and do the paperwork until the deal is made. Speaking of paperwork, our personnel department will take care of your legal requirements including visas, registration, health insurance and family matters e.g. advising on types and availability of day care centers, kindergartens and schools for your children.

If you come from outside the German speaking world, you will find that English is sufficient to get along in Vienna. For most people, English is actually part of their daily lives, due to an enormous exposure of the city to tourism. However, if you want to learn German you will be able to attend courses at the IMP's expense. Apart from sponsoring your language skills, the institute subsidizes sporting activities for its members.

More information about career opportunities at the IMP is available at: http://www.imp.univie.ac.at



POSTDOC IMPRESSIONS

"I wanted to widen my horizons, to see more of the world, but without endangering my scientific career. I think I have accomplished that by joining the IMP. I never regretted the decision and would encourage future postdocs - who are adaptive, open-minded and flexible - to look into opportunities abroad. The scientific atmosphere of the IMP is very exciting. I could have gone to other labs - in the US and in Europe - but I chose Kim's lab at the IMP because of the quality of science done here and the excellent facilities. The location in Vienna is of course the big bonus."

Rosemary Clyne, USA

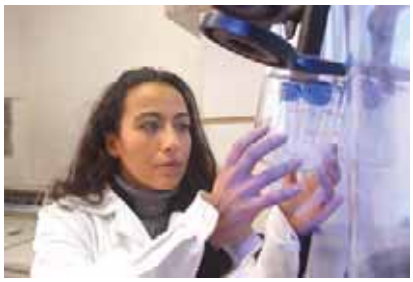
(Postdoc in Kim Nasmyth's lab)

"Like most of my friends, I was a bit prejudiced before I came to Vienna, thinking that Austrians in general are very conservative and locked up in history. As it turns out, I really enjoy living here. The contrast of old and modern, traditional and innovative, is a very refreshing experience. The prices are moderate and I can even afford tickets to world class concerts and to the opera. Most important though: Vienna is a very safe place to live in. This is something that my family and I will miss when we move on. "

Takashi Suzuki, Japan

(Postdoc in Barry Dickson's lab)

"The IMP's size is a positive asset in my opinion. The dimensions are not too large which makes it easy to keep an overview. We all know each other and we can all talk to each other. I would say, there is a human dimension to the institute. Whenever I need something from another group, the colleagues are helpful. There is this "open freezer-attitude", as I like to call it. Plus the Scientific Services are very efficient."



Latifa Bakiri, France

(Postdoc in Erwin Wagner's lab)

"The research here is excellent and the budget is generous, too. Accordingly, the expectations are very high as well. I would say, if you are highly motivated, the IMP is an ideal starting point for a career in science. As far as day-to-day life is concerned, I like the flat organizational hierarchy and the open doors philosophy. The contact to Boehringer Ingelheim is certainly an added bonus. As for Vienna, the cultural spectrum it offers is hard to beat. At the same time, I find it less hectic and less crowded than other cities of comparable size. What really strikes my family and me is how clean and safe the city is. I hope it stays that way."

Antoine Peters, Netherlands

(Postdoc in Thomas Jenuwein's lab)

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Gabi LITOS / Technician
Andreas SOMMER / Technician
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ONCOGENESIS: ABNORMAL DEVELOPMENTAL PLASTICITY?

In the development of cancer, e.g. leukemias and breast carcinomas, combinations of oncogenic and normal receptors/signal transducers are crucially important as they stimulate proliferation/renewal and inhibit differentiation/ apoptosis of primitive progenitors. We use novel in vitro cell culture systems and genetically modified mice to address the role of such signal combinations in leukemic and stress-induced renewal of hematopoietic progenitors, and in epithelial/mesenchymal transitions during metastasis and tissue remodeling.

Stress-induced alterations of renewal in hematopoietic progenitors: a process important in leukemogenesis

Renewal (i.e. sustained progenitor proliferation without differentiation) of primary erythroid progenitors reflects their physiological response to stress erythropoiesis. EpoR, c-Kit and the glucocorticoid receptor (GR) cooperate in renewal and require signaling through the Stat5 and PI3K pathways. In erythroleukemia, oncogenic receptor tyrosine kinases (RTks; v-ErbB, v-Sea) substitute for stress-induced signaling *via* EpoR/c-Kit, by activating both Stat5 and PI3K on their own. Erythroblasts from mutant mice lacking components of signaling complexes driving renewal (GR-/-, Stat5-/-, Raf-/-, Btk/Tec-/-) showed interesting defects in renewal and/or terminal differentiation (Figure 1). In addition, red cell differentiation proceeded as a cell-autonomous default program if apoptosis was prevented by the Epo-target gene Bcl-X_L. Finally, the human leukemia oncogene MLL (related to the *Drosophila* chromatin modifier *trithorax*) required cooperation with c-Kit to transform lymphohematopoietic cells and to cause multilineage leukemia.

We increasingly focus on investigating multipotent progenitor cell systems, and will further analyze interesting erythroid cell defects from respective knock-out mice. We have begun to explore hematopoietic progenitors from murine embryonic stem (ES) cells, a system suitable for characterization of possible hematopoietic defects, also in early embryonic lethal mouse mutants. We will address (also by RNAi) the function of a novel proapoptotic protein (p12) in the mitochondrial apoptosis pathway and its role in hematopoietic cells. We will also analyze cooperation between the GR and c-Kit/EpoR by expression profiling and in mice lacking Stat5, GR or both, and the composition/function of the EpoR/c-Kit- signalosome (Figure 1) in oncogene transformed cells.

Cooperation of RTK- and TGFβ-receptor signaling in tumor cell invasiveness and metastasis

Cooperation of a hyperactive Ras-MapK pathway with TGFβ-signaling causes epithelial/mesenchymal transition (EMT) in polarized mammary epithelial cells (EpH4), a truthful *in vitro* correlate of carcinoma cell metastasis. TGFβ induces Ras-transformed EpH4 cells

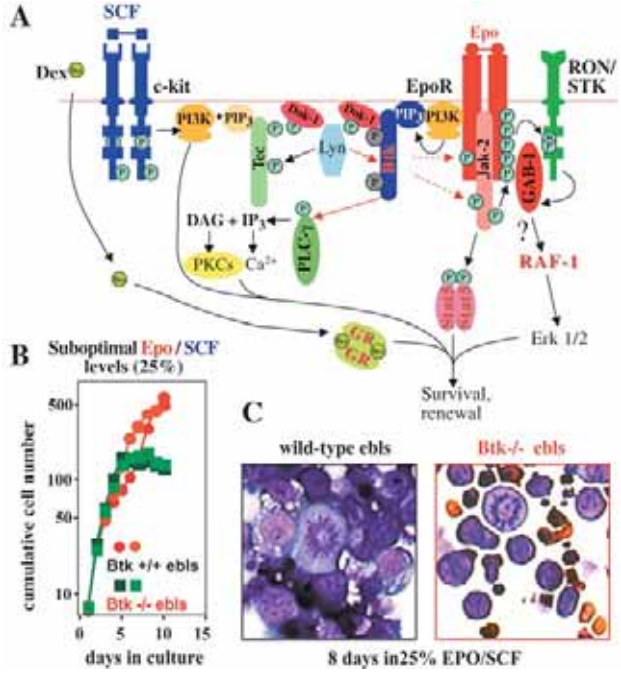

Figure 1: The EpoR / c-Kit signalosome: functional analysis in cells lacking specific signal transducers. (A) Highly simplified scheme of the EpoR/c-Kit signaling complex is shown, consisting of the EpoR, the RTKs Ron and c-Kit, numerous scaffolds (Gab1, Gab2, Doc) and PH-domain proteins e.g. the cytoplasmic tyrosine kinases Btk and Doc, plus further downstream signaling pathways.
(B, C) Erythroblasts lacking Btk (Btk -/-) show premature loss of proliferative potential in sub optimal (physiological) concentrations of Epo and SCF (B), caused by premature differentiation (small, brownish erythrocytes) under renewal conditions (C).

(EpRas) to undergo EMT, i.e. to acquire fibroblastoid morphology/increased migration and to undergo major reprogramming of gene expression towards mesenchymal cells. TGFβ plus Ras-activated PI3K signaling induces “scattering” (transient migratory phenotype without reprogramming of gene expression) *in vitro*, and causes tumor development, but not EMT or metastasis *in vivo*. Ras-dependent hyperproliferation of tumor cells *in vivo* could be recapitulated in 3D collagen cultures and was shown to require PI3K signaling (Figure 2). By expression profiling, we have identified multiple genes and pathways as candidates for having a crucial role in EMT/metastatis; e.g. we have found that TGFβ induces a PDGF-autocrine loop which is required for both, induction and maintenance of EMT.

We will increase our efforts to employ *in vivo* mouse models for mammary carcinoma progression and metastasis. Crossbreeding of transgenic MMTV-HER2- and MMTV-TGFβ1-transgenic mice already revealed strongly enhanced lung metastasis as compared to MMTV-HER2 mice, and EMT in the respective mammary tumors. Conversely, crosses between MMTV-HER2- and -dnTGFβRII transgenic mice are being examined for suppression of metastasis. This analysis will be extended to available knock-out mice lacking verified candidate genes from expression profiling (e.g. the E-cadherin repressor ΔEF-1, or the TGFβ co-receptor endoglin/CD105). Functional characterization of other EMT-specific genes, initially based on the RNAi approach, is also in progress. The kinetics of EMT in EpRas cells will be analyzed by expression profiling, after developing suitable methods. Furthermore, we are currently analyzing the possible cross talk between TGFβ- and Wnt/β-catenin signaling, since Smad2 mutants defective for the Smad/Lef-1 interaction abrogate both EMT and metastasis. Finally we will address recently uncovered cooperations between different TGFβRI family members (Alk-1/Alk-5), and ask whether cooperation between TGFβ and BMP signaling is relevant for EMT/metastasis.

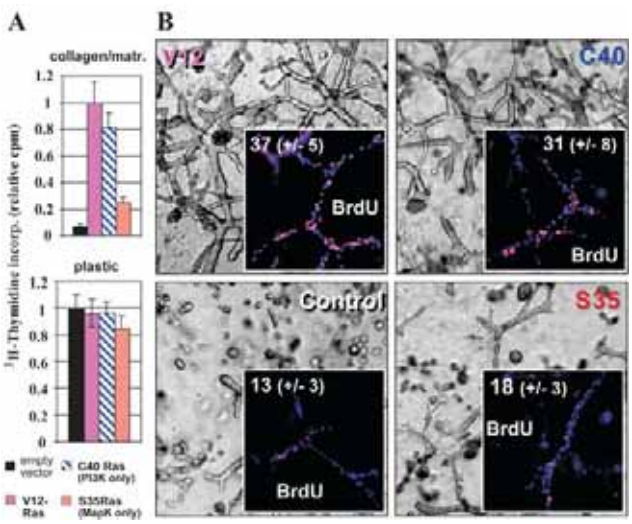

Figure 2: Hyperproliferation of Ras-transformed carcinoma cells driven by PI3K signaling in 3D cultures. (A) EpH4 cells expressing unmutated oncogenic Ras (V12Ras), effector-specific Ras mutants selectively activating either the MapK pathway (S35-Ras) or the PI3K pathway (C40-Ras) and empty vector control cells were seeded in serum-free collagen gels (3D cultures, top) or on plastic (bottom) and analyzed for [3H]-thymidine incorporation. Ras-driven hyperproliferation (as in tumors *in vivo*) is seen only in collagen gels and is dependent on Ras-driven, hyperactive PI3K signaling.
(B) Photographs of collagen gel structures from the same cells as in A, before (bright field) or after staining for incorporated BrdU (insets, pink nuclei), showing again PI3K-dependent enhanced proliferation.

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STEM CELL COMMITMENT IN HEMATOPOIESIS AND ORGANOGENESIS

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

Hematopoiesis

A fundamental question in hematopoiesis is how stem cells and early progenitors become committed to a single developmental pathway and then differentiate into mature cell types of the selected lineage. By analyzing the transcription factor Pax5, we have gained insight into the commitment process of the B-lymphoid lineage. *Pax5* is essential for the progression of B cell development beyond an early progenitor (pro-B) cell stage. Pax5-deficient pro-B cells can be cultured *ex vivo* on a layer of stromal cells in the presence of IL-7. However, these pro-B cells are uncommitted progenitor cells, as they can develop *in vitro* and *in vivo* into various hematopoietic cell types except for B cells, which are only generated upon retroviral restoration of *Pax5* expression (Figure 1). Pax5 has thus been identified as the B-lineage commitment factor, which restricts the developmental potential of progenitor cells to the B cell pathway. Conditional gene inactivation revealed that *Pax5* expression is continuously required to maintain B-lineage commitment, as its loss converts committed B-lymphocytes into early hematopoietic progenitors with multilineage potential. Pax5 therefore controls the identity of B-lymphocytes throughout B cell development, which raises the question about the upstream transcription factors regulating *Pax5* expression. Using transgenic approaches, we have mapped the B-cell-specific enhancer of the *Pax5* locus, which will now facilitate identification of the upstream regulatory factors.

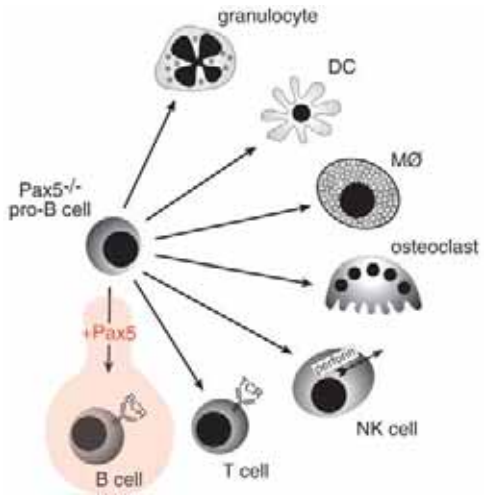

Figure 1: B-lineage commitment by Pax5. *Pax5*$^{-/-}$ pro-B cells are early progenitor cells, which can differentiate along the indicated hematopoietic lineages with the exception of the B cell pathway.

At the molecular level, Pax5 fulfils a dual role by activating the expression of B-cell-specific genes and by repressing the transcription of lineage-inappropriate

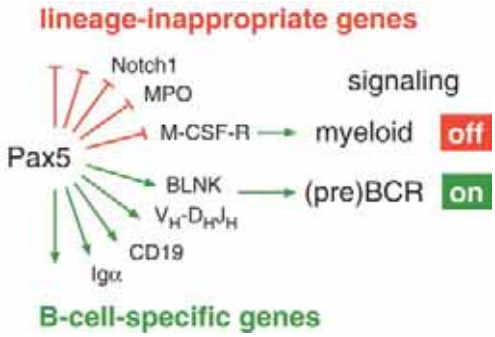

Figure 2: Dual role of Pax5 in B-lymphopoiesis. Pax5 activates B-lymphoid genes (green) and simultaneously represses lineage-inappropriate genes (red).

genes. To systematically analyze the transcriptional function of Pax5, we have used cDNA microarray screening to identify a multitude of novel Pax5-regulated genes. One of the activated Pax5 target genes codes for the central adaptor protein BLNK, which couples signaling from the (pre)B cell receptor to transcriptional changes in the nucleus (Figure 2). The majority of identified genes are, however, repressed by Pax5. These genes are normally expressed during erythroid, myeloid or T-lymphoid differentiation, demonstrating that the Pax5-deficient progenitors promiscuously express genes from different hematopoietic lineages. We are currently testing the hypothesis that this promiscuous gene expression is responsible for the developmental plasticity of early progenitors.

We have also investigated the lineage commitment function of Pax5 by forced expression in hematopoietic stem cells and early progenitors. Pan-hematopoietic *Pax5* expression strongly promotes B cell development at the expense of T-lymphopoiesis, whereas myeloid and erythroid development is only minimally affected. Pax5 thereby interferes with T-lineage commitment and early thymocyte development by directly repressing the transcription of the T cell specification gene *Notch1* (Figure 2). This ectopic expression system allows us now to study the role of Pax5 in controlling V(D)J rearrangements at the immunoglobulin heavy-chain locus in heterologous T cells.

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Midbrain and kidney development

The midbrain and cerebellum develop from an organizing center at the midbrain-hindbrain boundary (MHB), which secretes the signaling molecule Fgf8. The three transcription factors of the Pax2/5/8 family are co-expressed in this embryonic brain region. Gene targeting and transgenic analyses have demonstrated that Pax2, the earliest marker of the MHB region, is essential not only for *Pax5* and *Pax8* expression, but also for activation of the organizer signal Fgf8 itself. BAC transgenic analysis has identified the transcription factor Oct3/4 as an upstream regulator of the early MHB-specific enhancer of *Pax2*. Consequently, MHB development is controlled by the following regulatory cascade: Oct3/4 ➧ Pax2 ➧ Fgf8. Pax2, together with Pax8, is also the earliest marker of kidney development. We have shown that Pax2 and Pax8 together are essential for the commitment of intermediate mesoderm cells to the nephric lineage, as *Pax2*, *Pax8* double mutant embryos fail to form even the pronephros – the earliest stage of kidney development (Figure 3A, B). Conversely, retroviral misexpression of *Pax2* is sufficient to induce ectopic nephric structures in the intermediate mesoderm (Figure 3C). We are currently characterizing novel Pax target genes involved in the specification of MHB and kidney development.

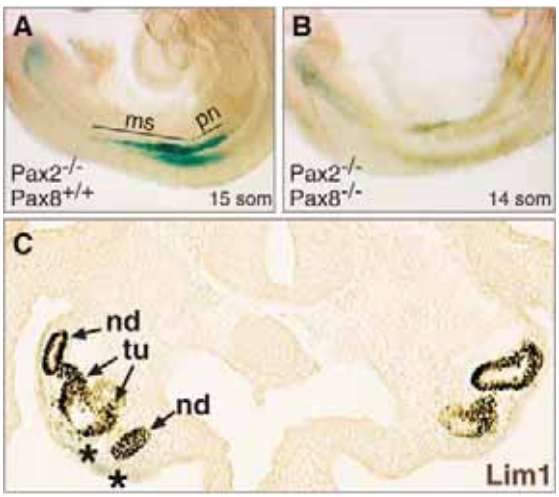

Figure 3: Nephric lineage specification by Pax2 and Pax8. (A, B) The expression of β-galactosidase from the *Pax2* mutant allele was used as a kidney-specific marker to visualize the development of the pronephros (pn) and mesonephros (ms) in mouse embryos of the indicated genotypes. (C) Formation of an ectopic kidney (asterisks) in chicken embryos that were injected with a Pax2-expressing retrovirus. Lim1 staining identifies the tubules (tu) and nephric duct (nd) of the developing kidney.

Tim CLAUSEN[1] / Group Leader

Boril BOYANOV / PhD Student
Tobias KROJER[2] / PhD Student
Corinna WILKEN / PhD Student
Robert KURZBAUER / Technician

[1]since November 2002, [2]Max Planck Institute, Biochemistry, Germany

MOLECULAR MECHANISMS OF PROTEIN QUALITY CONTROL

Cells precisely monitor the concentration and functionality of each protein for optimal performance. This quality control is achieved by a sophisticated interplay of proteases and molecular chaperones. We are performing a structure-function analysis of the heat-shock protein DegP (HtrA), which combines both refolding and digesting activities and thus offers unique possibilities to investigate how cells distinguish non-native proteins that can be refolded from hopeless cases that have to be digested.

Damaged proteins represent a serious hazard to the cell as they might accumulate as large aggregates, a process associated with prion and other amyloid diseases. *In vivo*, aggregate formation is a highly favorable process due to the extremely high intracellular protein concentrations (100-150 mg/ml). DegP is a protease-chaperone that aims to reduce the amount of unfolded protein. This heat-shock protein was initially identified in *Escherichia coli* by two phenotypes of corresponding null mutants and named accordingly. Mutants either did not grow at elevated temperatures (HtrA, High temperature requirement) or failed to digest misfolded proteins in the periplasm (DegP, Degradation). The defining feature of the DegP family is the combination of a trypsin-like protease domain with at least one C-terminal PDZ domain, a prominent protein-protein interaction motif. Prokaryotic DegP has been attributed to the tolerance against various folding stresses as well as to pathogenicity. Human homologues are believed to be involved in arthritis, cell growth regulation, unfolded protein response and programmed cell death. Beside this physiological impact, the protein itself has unique mechanistic properties suggesting that DegP represents a novel protease-chaperone system.

To better understand its mode of action we have started a structural analysis of the well-characterized *E. coli* DegP protein. Central questions of interest include the ATP-independent mechanism of protease and refolding activities, analysis of the reversible, temperature-dependent switch from chaperone to protease, the function of the PDZ domains and the structural determinants of substrate specificity. The crystal structure of the chaperone form of DegP, which was recently determined in our laboratory, is a start in dissecting the molecular mechanism of this quality control factor, providing the required stereochemical framework for further genetic, biochemical, and biophysical studies.

The DegP hexamer is formed by staggered association of trimeric rings (Figure 1). The proteolytic sites are located in a central cavity that is only accessible laterally.

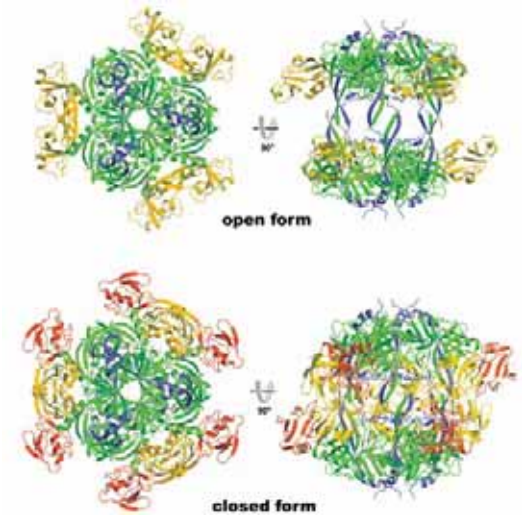

Figure 1: Top and side views of the two hexamers observed in the DegP crystals representing the open and closed form of the molecule. The individual domains are colored differently with the N-terminal domain in blue, the protease in green and the PDZ domains in yellow and red, respectively. Both hexamers are approximately equal in size having a height of 105 Å and a diameter of 120 Å.

The mobile sidewalls are constructed by twelve PDZ domains, which mediate the opening and closing of the particle and probably the initial interaction with substrate. Further binding sites for misfolded proteins are located within the inner cavity (Figure 2). Due to the geometric constraints of this chamber, substrates must be at least partially unfolded to enter. As in other chaperones of known structure, the DegP cavity is lined by hydrophobic residues. These residues form two binding platforms, which have a pronounced structural flexibility as judged from their high thermal motion factors. This plasticity should allow binding of diverse polypeptides.

Cage-forming proteases and chaperones can be energy-dependent or energy-independent. In the former group, ATPase activity is important for recognition of target proteins, their dissociation and unfolding, their translocation within the complex and various gating mechanisms. The present crystal structure indicates why these functions are not relevant for DegP. DegP preferably degrades substrates, which are *per se* partially unfolded and which might accumulate under extreme conditions. Alternatively, threading of substrate through the inner chamber could promote unfolding into an extended conformation. Removal of higher order structural elements would allow the substrate to reinitiate folding after exit from DegP. Recruitment of PDZ domains for the gating mechanism should permit a direct coupling of substrate binding and translocation within the DegP particle. Accordingly, the PDZ domains may function as tentacular arms capturing substrates and transferring them into the inner cavity. By binding to the C-terminus or a β-hairpin loop of a protein, the PDZ domains could properly position the substrate for threading it into the central cavity. After accessing this chamber, the fate of the unfolded protein depends on the interplay of several active site loops (loops LA, L1, L2, L3 using the protease nomenclature), which regulate proteolytic activity.

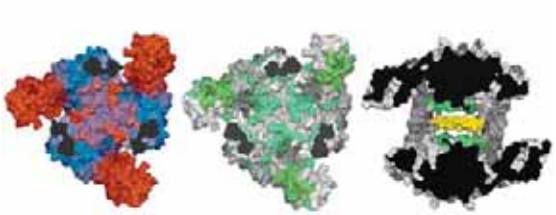

Figure 2: The internal cavity of DegP. For better illustration half cut figures of the molecular surface were prepared from both top and side views. The cutting area is shown in black. The chaperone-like features of the inner cavity are the following: (Left) Plasticity. Flexible portions are in red, whereas rigid areas are colored blue. (Middle) Hydrophobicity. The inner cavity is mainly constructed by hydrophobic residues, which are highlighted in cyan and green. (Right) Size exclusion. To illustrate the geometric restriction of the molecular compactor, a single α-helix (yellow) was modeled into the cavity.

Future studies will concentrate on the characterization of the protease form of DegP. We will aim to determine high-resolution crystal structures with substrates and inhibitors and extend the approach to the related DegQ and DegS proteases. The search for additional physiological substrates and for cellular effectors that either inhibit or activate members of the DegP family will contribute to our understanding of the fascinating network controlling protein composition, which is undoubtedly one of the key metabolic pathways of each cell.

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AXON GUIDANCE AND TARGET RECOGNITION

It is a fascinating but daunting problem: How do just a few thousand genes direct the assembly of neuronal circuits with such staggering complexity as those of the human brain? We chose to tackle the fly first. It's not that the fly's nervous system is any less challenging, but at least we have a powerful set of genetic tools to work with. And what we learn about the development of the fly's nervous system may provide new insights into our own. After all, it seems that there's a bit of a fly in all of us.

Neuronal circuits are formed as each neuron sends out axons and dendrites to find, recognize and connect with the appropriate target cells. Finding the target is the task of the growth cone, a highly motile and exquisitely sensitive structure at the tip of each axon and dendrite. Growth cones detect various guidance cues in the extracellular environment, and somehow manage to extract from this information the correct route towards their target. By studying this process in *Drosophila*, we hope to find out what these cues are, how growth cones detect them, and how each growth cone knows which cues it should follow and which it should ignore. Genetics is a powerful tool in this endeavor. Mutations that disrupt neuronal connectivity can lead us to the genes that encode the guidance cues themselves, or to the molecules that growth cones use to detect and respond to these cues. And, by examining how these molecules work *in vitro* and *in vivo*, we can begin to explore the molecular, cellular and developmental mechanisms that ensure that each growth cone responds to the right cues at the right time, and ignores the rest. We are focussing on two different systems: the ventral nerve cord of the embryo, and the adult visual system. Some of our recent findings and ongoing projects are highlighted here.

Axon guidance in the ventral nerve cord

In bilaterally symmetric nervous systems, such as our own and the fly's, axons in the CNS must decide whether or not to grow across the midline. Our recent work has revealed how this decision is controlled in the *Drosophila* nerve cord (Figure 1). Crossing and non-crossing axons differ in their sensitivity to the midline repellent Slit. Both commissural axons (which cross) and ipsilateral axons (which don't) express the Slit receptor Roundabout (Robo for short). In ipsilateral axons, Robo is inserted into the growth cone, making them sensitive to the repulsive activity of Slit. But in commissural neurons, Robo is not delivered to the growth cone until *after* it has crossed the midline. So these axons are able to cross, but only once. Before crossing, an intracellular sorting receptor called Commissureless (Comm) collects the newly synthesized Robo protein at the Golgi and delivers it to lysosomes, where it is degraded. Comm is normally active only in commissural neurons as their axons first

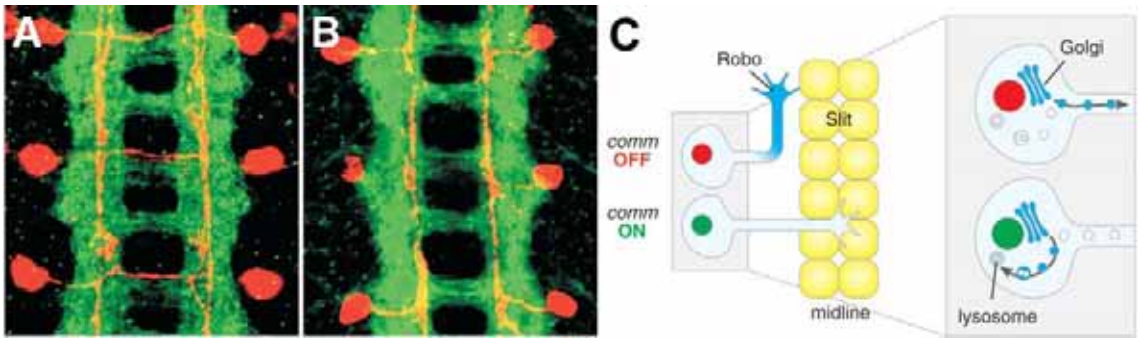

Figure 1: Crossing the midline. In the *Drosophila* CNS, some axons cross the midline (like the red axons in A), but others do not (B). Those that do cross, called commissural axons, cross only once. Comm functions as a switch to control this decision (C). Comm is ON in commissural neurons as they cross, but OFF in ipsilateral neurons and post-crossing commissural neurons. Comm regulates the sensitivity of axons to the midline repellent Slit (yellow in C). It does this by controlling the intracellular trafficking of Robo (blue), the Slit receptor.

grow toward and across the midline. If it is made inactive (by a mutation), no axon can cross the midline, resulting in the commissureless phenotype from which the gene got its name. In ipsilateral neurons, and post-crossing commissural neurons, Comm is normally inactive. But by activating Comm in these neurons, we can force them to cross (or recross). This defines Comm as a simple genetic switch to control midline crossing (Figure 1). We are currently trying to find out how this switch is turned ON and OFF, and how Comm selects Robo and sorts it to lysosomes.

Axon guidance in the visual system

Flies have excellent vision. This rests in part on the extraordinarily precise connections established between photoreceptors in the eye and their targets in the brain. As a first step in determining how these connections are established, we screened though more than 32,000 mutant lines to find some 200 mutants with abnormal connectivity patterns. These mutations define about 50 different genes, 31 of which we have now identified. These genes encode cell surface receptors and signaling molecules, as well as factors involved in gene transcription, axonal transport, and membrane trafficking. One of them encodes a 7-transmembrane cadherin called Flamingo. Flamingo is expressed on many different photoreceptor axons and their target cells in the brain (Figure 2). It is needed for one class of photoreceptors (R8s) to select their correct targets in a specific layer of the brain. We continue to look for the remaining genes, and are beginning to piece together the molecular pathways and processes that underlie this exceptional example of neuronal engineering.

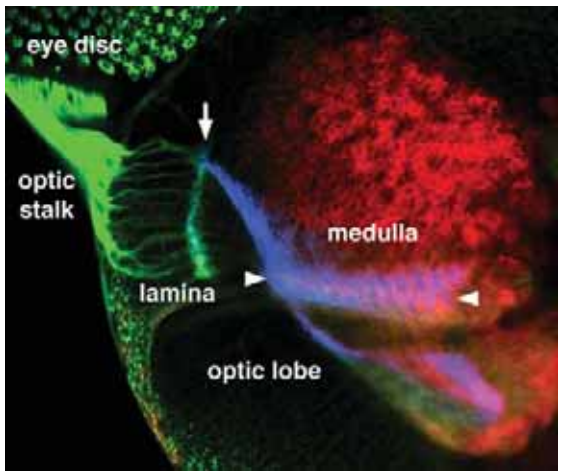

Figure 2: Photoreceptor axon targeting. Photoreceptor axons (green) extend from the eye disc, through the optic stalk, and into the optic lobe. Some axons terminate in the lamina (arrow). Others, including R8 axons, terminate in the medulla (arrowhead), where they form synapses with medulla neurons (red). The Flamingo protein (blue) is required for correct targeting of R8 axons.

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UNDERSTANDING MOLECULAR MECHANISMS WITH BIOMOLECULAR SEQUENCE ANALYSIS

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

Application projects in cooperation with experimental groups

The creation of an efficient environment for using biological databases and sequence analysis software in applied projects is the most important technical achievement of the Bioinformatics unit. A number of these services are available not only *via* command line within the Bioinformatics group net but also through the local Intranet and Internet node (http://mendel.imp.univie.ac.at).

Many genetic screens and cDNA chip studies end up in sequences of functionally uncharacterized biomolecules. In such situations, sensitive sequence analyses may produce crucial insights. More than 200 gene or protein families have been studied in great detail during the past year, some of them repeatedly, to elucidate structural and molecular functional features of the gene products or associated genomic regulatory regions. Such investigations have been launched, as a rule, on requests of IMP researchers and their Austrian and international collaborators.

For example, the putative acetyltransferase activity of eco1p proteins has been predicted by a distant, statistically not significant homology of their C-terminal domain segment with histone acetyltransferases. Additional support was obtained from structural consideration - the conservation of residues that form the CoA binding pocket and from the conservation of predicted secondary structure (Figure 1). Subsequent experiments carried out by the Nasmyth group successfully verified this hypothesis and found cohesion complex components and pds5p as possible substrates.

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

Genuine bioinformatics research is oriented on the creation of new methods or integrative theories but scientifically relevant directions of such efforts are

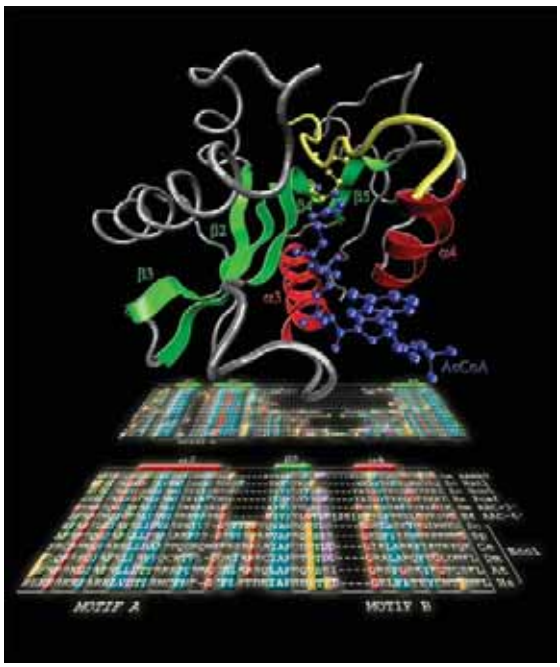

Figure 1: Eco1p - an acetyltransferase in cohesion. The image is an artist's view of how our understanding of protein structure and function is emerging from a sequence alignment. It shows the joint multiple alignment of Eco1 sequences from different organisms and sequences of members of the GNAT superfamily of acetyltransferases. The alignment was obtained based on domain fragment searches, secondary structure predictions and physico-chemical similarity of amino acid types. The three-dimensional structure of GCN5 histone N-acetyltransferase from *Tetrahymena thermophilum* [Rojas et al. Nature 1999; 401:93-98] is shown above. The secondary structure elements of the most conserved motifs D, A, and B that were predicted in Eco1 are colored. Eco1 is a protein that has been previously implicated in the establishment of bridges between sister chromatids during DNA replication. The unexpected acetyltransferase activity of Eco1 is reported in the paper by D. Ivanov et al. (Curr Biol. 2002, 12(4):323-328).

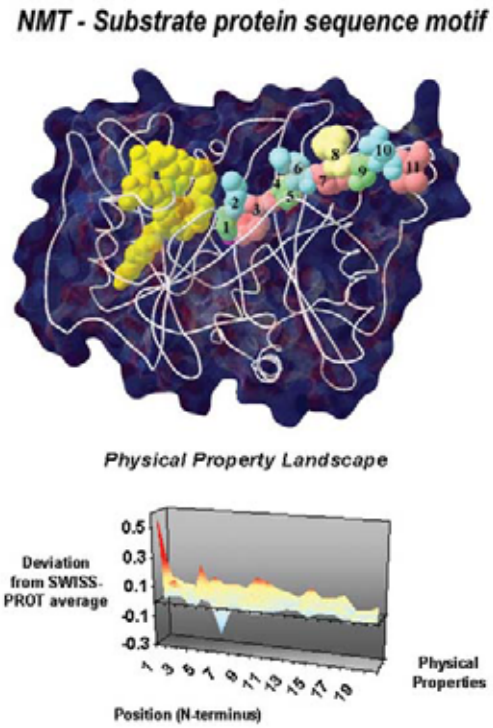

Figure 2: The N-terminal N-myristoylation of proteins. Myristoyl-CoA: protein N-myristoyltransferase (NMT) recognizes the N-termini of various eukaryotic and viral proteins and attaches myristate as lipid anchor to direct them to diverse membranes. We refined the sequence requirements for substrate recognition and described the motif also in terms of characteristic deviations of physical properties from random proteins (SWISSPROT average). Transforming our knowledge into a scoring function, we were able to build a predictor for this lipid modification whose selectivity and sensitivity allows proteome-wide database annotations.

determined by interaction with experimental life sciences. Our methodical research has been grouped around two main lines:

1. Recognition of posttranslational modification signals in protein sequences. We have developed a myristoylation predictor (Figure 2).
2. Integration of diverse sequence analysis methods in a higher order shell ("automatic sequence analyzer") for applications in large-scale protein sequence annotation; a common project with Boehringer-Ingelheim Austria.

Computer usage and networking at the IMP

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

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THE MECHANISM OF CYTOKINESIS

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

Cytokinesis is mediated by an actin-based contractile ring that is attached to the overlying cell membrane. The ring assembles in the cell cortex at a site that is positioned midway between the two poles of the mitotic spindle ensuring that the two separated sets of chromosomes are equally partitioned into the two daughter cells. The entire process - assembly of the contractile ring, its constriction, and the separation of the two nascent cells - typically requires ten minutes. Thus, cytokinesis is a dynamic and spatially regulated process. We are using the nematode *Caenorhabditis elegans (C. elegans)* as a model system to dissect this complex process since worm embryos are extremely well suited for real time microscopic analysis (Figure 1). Furthermore, this system can be molecularly dissected using forward and reverse genetics to address the following unsolved problems: How is the cleavage furrow positioned? How does the contractile ring assemble and function? How does the central spindle assemble and function? How is completion of cytokinesis achieved?

We are particularly interested in the assembly and function of the central spindle, which arises from a subset of the microtubules that make up mitotic spindle.

Figure 1: The first division of a worm embryo.

Michael Glotzer

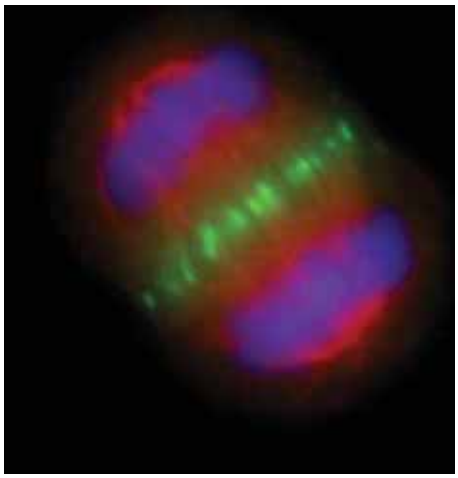

Figure 2: Centralspindlin localizes to the spindle midzone in anaphase. A mammalian cell in anaphase has been stained for MKLP-1 (the mammalian ZEN-4 ortholog; green), tubulin (red) and DNA (blue).

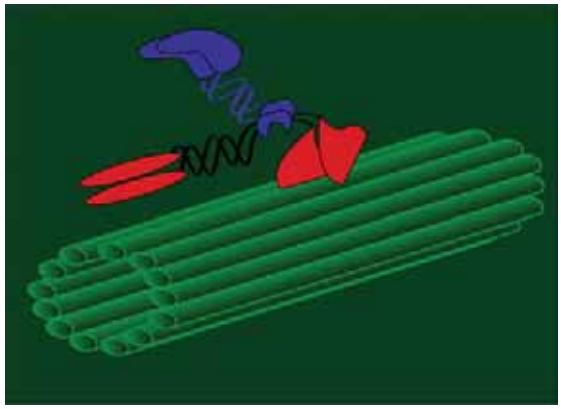

Figure 3: A schematic model of the centralspindlin complex. The interacting domains of CYK-4 (blue) and ZEN-4 (red) have been defined and the nature of the complex studied with biochemical techniques. Based on these data, we have proposed this working model of centralspindlin bound to a microtubule (green).

Central spindle assembly begins at the metaphase to anaphase transition, when chromosomes move polewards on the shrinking kinetochore microtubules. At this time, spindle microtubules become bundled to form the central spindle (Figure 2). We have found an evolutionarily conserved protein complex, the centralspindlin complex, consisting of a Rho family GAP, CYK-4, and a kinesin like protein, ZEN-4, that is directly involved in central spindle assembly (Figure 3). Embryos deficient for CYK-4 or ZEN-4 are defective in both central spindle assembly and cytokinesis. CYK-4 contains a GAP domain that stimulates GTP hydrolysis by Rho-family GTPases. It is likely that CYK-4 promotes the completion of cytokinesis by virtue of its ability to promote GTP hydrolysis by Rho. We have reconstituted centralspindlin-mediated microtubule bundling *in vitro* and are using this system to understand how this structurally unique kinesin-like protein functions at the molecular level and how it is regulated in both space and time.

A second protein that localizes to the central spindle is the Aurora-B like kinase AIR-2. In previous years we demonstrated that Aurora-B binds to ICP-1 (the nematode Incenp) and that ICP-1 is required for localization of this kinase. Embryos lacking AIR-2 and ICP-1 are defective in both chromosome segregation and cytokinesis. We have recently identified another member of this kinase complex, CSC-1, which forms a complex with ICP-1 and the survivin-like protein BIR-1. We have reconstituted this tetrameric kinase complex with recombinant proteins and are investigating its regulation. ICP-1 and AIR-2 promote the stable localization of ZEN-4 to the central spindle. We are investigating whether ZEN-4 or CYK-4 is a direct substrate of the AIR-2 kinase and if so, how phosphorylation affects the activity of the centralspindlin complex.

The analysis of cytokinesis in *C. elegans* embryos lacking a central spindle reveals that this structure is not required at the early stages of cytokinesis. However, the central spindle does appear to have a critical role in furrow formation in other systems. We have recently gained insight into this apparent discrepancy. We have found that the central spindle is required for furrow initiation even in *C. elegans* embryos, but only under certain conditions. We are further studying the properties of the mitotic spindle that underlie its ability to induce a single, precisely positioned, cleavage furrow and are beginning to dissect the molecular basis of this process.

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FORMATION AND PATTERNING OF THE VERTEBRATE SKELETON

The skeleton is an important structure of the vertebrate organism; it supports the body, provides the mechanical framework for physical movements, and protects internal organs. To perform these vital functions, bone and cartilage must form in an exact pattern, with each skeletal element attaining its proper relative length and shape, and each articulation forming precisely between adjoining elements. We are using mouse and chick as model organisms to gain insight into how these different processes are regulated and coordinated during embryonic and postnatal development.

Regulation of chondrocyte maturation and bone homeostasis

Using a gain-of-function approach we have shown that in chick three *Wnt* genes, *Wnt4*, *5a*, and *5b*, are involved in regulating chondrocyte maturation. While Wnt5a and 5b, serve as negative signals, Wnt4 acts as a positive signal in chondrocyte maturation and, in addition, in bone collar differentiation (Hartmann and Tabin, 2000; unpublished observation). How is this specificity achieved? We have obtained evidence that the different Wnts utilize distinct intracellular signaling pathways; Wnt4 signals through the canonical Wnt pathway, while, Wnt5a/5b do not. We are currently investigating which pathway is utilized by Wnt5a/5b. Our long-term interest is to understand the fine tuning mechanisms enabling coordinated growth of skeletal elements. We would like to uncover cross talk between different regulatory signals controlling chondrocyte maturation. Therefore, we are currently also investigating other factors affecting chondrogenesis.

Although all, Wnt4, 5a and 5b, are expressed in chondrogenic regions in mouse, changes in cartilage maturation have only been observed in Wnt5a loss-of-function mutants. Interestingly, in the mouse the expression domains of Wnt4, 5a and 5b overlap in the growth plates of long bones that differs from their expression pattern in chick. Somewhat unexpectedly, the analysis of various double mutant combinations has not revealed any obvious defects in skeletal maturation (work done in collaboration with Dr. McMahon's lab at Harvard University). Consequently, we are now addressing whether the canonical Wnt-pathway plays

Figure 1: Skeleton of a newborn mouse. Cartilagenous regions are stained blue; bone is stained red.

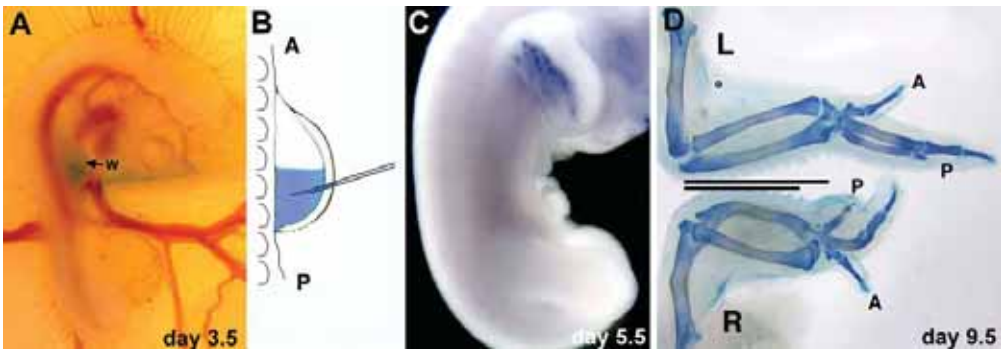

Figure 2: *In ovo* gain-of-function experiment with chick using retroviral injections to investigate the potential role of different factors in skeletogenesis. (A) Injections of retroviral particles are performed at day 3.5 of chick embryonic development into the posterior half anlage of the future wing (w). (B) Schematic drawing of the injected limb bud (injected region is colored blue). (C) Visualization of the infection two days after the injection, shown by the blue staining. (D) Visualization of morphological changes six days after the injection, showing the effects of the gain-of-function experiment on the skeletal elements of the right (R) wing while the left (L) wing is unaffected (cartilage elements are stained blue).

a role in mouse skeletogenesis at all using a conditional gene targeting approach. Recent reports have implicated Wnt-signaling in the control of bone development and maintenance (Hartmann and Tabin, 2000; Kato et al., 2002). In particular, we are interested in analyzing potential roles of Wnt4 and Wnt14 in bone homeostasis using both, chick and mouse.

Synovial joint development

Our recent analysis of the role of Wnt14 in chick skeletogenesis has identified Wnt14 as a major player in the induction process of synovial joint development (Hartmann and Tabin, 2001). However, in various cell culture systems, Wnt14 alone is unable to induce the same responses as *in ovo*. Thus, we are trying now to establish an *in vitro* co-culture system in order to identify co-factors required for the induction of markers characteristic of the early joint interzone.

We are also investigating whether Wnt14 is necessary for joint development in the mouse model system. In addition, since Wnt14 continues to be expressed in synoviocytes of the mature joint, we would like to determine whether Wnt14 plays a role in maintaining joint integrity. This late expression of Wnt14 is very interesting in light of joint diseases associated with alterations of the synovium, such as rheumatoid- or osteoarthritis. We are currently addressing potential late function(s) of Wnt14 in the joint using gain- and loss-of-function experiments.

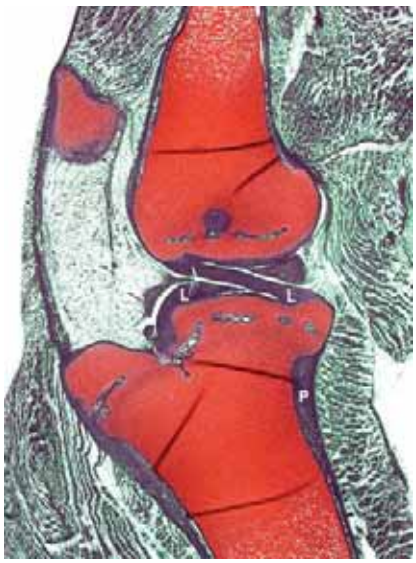

Figure 3: Section through the joint of a chicken knee. Cartilage is stained in red, soft tissue is stained greenish, perichondrium (P) and ligaments (L) in the joint are stained in dark blue.

Our long-term goals are to identify regulators as well as target genes of Wnt14. Using a transgenic approach in combination with searching for evolutionary highly conserved genomic regions within the Wnt14 locus we are attempting to identify regulatory elements responsible for the expression in the early joint interzone. The identification of such a joint specific element will provide a useful tool to address the question which factors are necessary for initiation and regulation of Wnt14 in the joint forming region and will inevitably allow us to understand how the skeleton is patterned.

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EPITHELIAL BIOLOGY

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

In our research we use well-characterized cell lines that display both, epithelial polarity, and its cancer-like disruption. A mouse mammary gland epithelial cell line (EpH4) expresses an estrogen-inducible c-JunER fusion protein that allows analysis of reversible loss of epithelial polarity. This hormone-induced disruption of epithelial polarity in EpH4 cells entailes loss of trans-epithelial resistance, redistribution of both apical and basolateral proteins over the entire plasma membrane and destabilization of junctional complexes. Using this cell system, we aimed to identify genes and proteins that are differentially expressed during loss of epithelial polarity. First, we screened for genes differentially expressed during loss of epithelial polarity. Second, we analyzed differentially expressed proteins on subcellular organelles by proteomics techniques. During the past two years we focused on the analysis of candidate genes derived from those screens:

Endosomal MP1 - MAPK scaffold complex

Although various signals are routed through the same extra-cellular signal regulated kinase (ERK) cascade, cells discriminate among the stimuli and respond accordingly. To regulate proper signal transduction, specific signaling units are organized, that employ a variety of scaffolds and adaptors. Last year we discovered a novel highly conserved protein of 14 kD that is peripherally associated with the cytoplasmic surfaces of late endosomes/lysosomes. We identified MP1 (Mek1 partner), a catalytic scaffold protein of the MAPK-pathway, as p14 interacting protein. The scaffold protein MP1 assembles a specific scaffold-complex in the ERK cascade. This year we have shown, that p14 is an endosomal adaptor protein for MP1 and is required for MAPK signaling. Reduction of p14 protein levels by siRNAi results in defective signal transduction as well as in translocation of MP1 from the endosomes to the cytoplasm . Therefore, our results suggest that the p14-dependent intracellular localization of the MP1 scaffold complex to endosomes is essential for signal transduction.

We are investigating this possible role of p14 in *Drosophila* using reversed genetics. In addition, we are trying to interfere with p14 expression by inducible RNAi. p14 knock-down causes lethality in the pupal stage and preliminary analysis shows a severe defect in thoracic bristle development, a phenotype previously reported for hypomorphic mutations in the EGFR pathway (Culi et al. 2001). Conversely, overexpression of p14 in the peripheral nervous system results in the development of ectopic macrochaetea on the fly notae, which is consistent with an over-activation of the MAPK pathway.

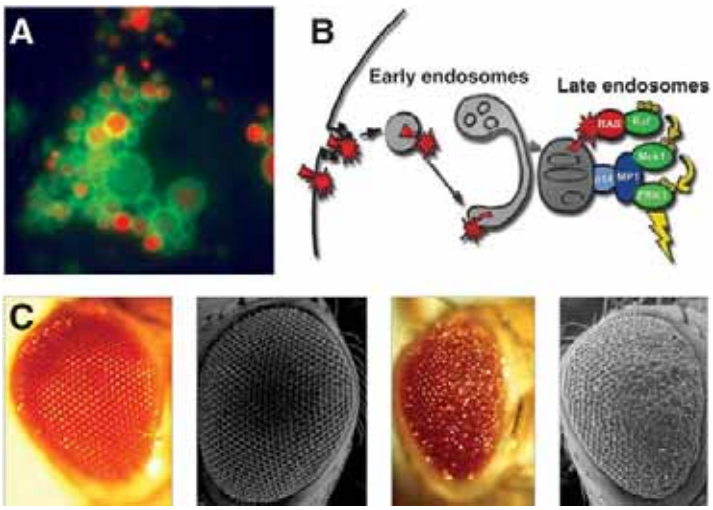

Figure 1: (A) EGF-rhodamine was internalized for 24 hrs in Caco-2 cells, stably transfected with EGFP-p14. Living cells were observed under a confocal microscope. After 24 hrs internalization the EGFP-p14 containing comparment was filled with EGF-rhodamine.
(B) Schematic representation of the intracellular routes taken by the activated EGF-receptor and the p14/MP1/MAP kinase complex on late endosomes.
(C) Overexpression of p14 in the adult eye of *Drosphila*. Left pair of panels (light microscopy/scanning electron microscopy): wild type; right pair of panels: Sev-Gal4/UAS-p14.

In addition, ectopic expression of p14 in photoreceptor cells results in rough eyes (Figure 1), and, using antibodies against phosphorylated Erk we could correlate this phenotype with an over-activation of MAPK.

TIS7 and SIN3 HDAC complex

Mammalian histone deacetylases HDAC1 and HDAC2 are members of the multisubunit SIN3 complex, containing also transcriptional co-repressors Sin3A and Sin3B, nuclear co-receptor N-CoR, several associated polypeptides like SAP30, and other, so far unidentified proteins. This year we have shown that the mouse TIS7 protein is a novel transcriptional co-repressor that associates with the SIN3 complex. We have identified TIS7 as a gene that is upregulated upon loss of polarity in EpH4 cell line - TIS7 protein levels increase and the protein translocates into the nucleus. Overexpression of TIS7 causes loss of polarity and represses a restrictive set of genes, as revealed by cDNA microarray analysis. To investigate the molecular mechanism of TIS7-mediated transcriptional repression, yeast two-hybrid screen and co-immunoprecipitations were performed. In EpH4 cells, TIS7 protein interacts with several proteins of the SIN3 complex, including mSin3B, the histone deacetylase HDAC1, nuclear receptor co-repressor N-CoR and SAP30. The co-immunoprecipitated HDAC complex is enzymatically active. TIS7 targeted to a GAL4-dependent reporter represses transcription. Finally, we have demonstrated that the transcriptional repression of endogenous genes by TIS7 overexpression is HDAC dependent. Thus, we propose that TIS7 is a transcriptional co-repressor affecting the expression of specific genes in a HDAC activity-dependent manner during cell fate decisions, e.g. scattering. Currently, we are functionally characterizing a gene highly related to TIS7 that we have recently cloned, mSKMC15, which has 88% homology with TIS7 at the amino acid level. We have embarked on the knockout of both genes in mice.

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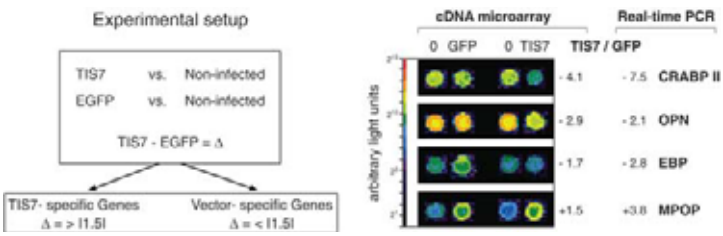
Figure 2: Identification of TIS7 downstreamn target genes by cDNA microarray analysis: genes regulated differentially by TIS7 or the vector CELO, and vector-associated alterations only. All expression data can be viewed and downloaded *via* the web-browser at http://www.imp.univie.ac.at/lh/chip_10_5_01/index.html. On the left, the intensities of hybridization signals from control (0), Celo-eGFP and Celo-TIS7 infected cells are shown, respectively. On the right, the ratio between the signal in Celo-TIS7 and Celo-eGFP samples is shown. The results were confirmed using "Light cycler" real-time PCR analysis. CRABP II, cellular retionic acid binding protein; OPN, osteopontin; EBP, emopamil binding protein; MP0P, myelin protein 0 precursor.



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EPIGENETIC CONTROL BY MAMMALIAN HISTONE METHYL TRANSFERASES

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

Histone methylation and the biochemistry of heterochromatin

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

The many faces of H3-K9 methylation

In addition to its function in constitutive heterochromatin formation at pericentric regions, the SUV39H1 HMTase is also involved in local gene repression and is targeted to specific cell cycle genes through the tumor suppressor Rb. Further, H3-K9 methylation also occurs at facultative heterochromatin of the inactive X chromosome in female mammals. H3-K9 methylation is retained through mitosis, indicating that it could provide an epigenetic imprint for the maintenance of

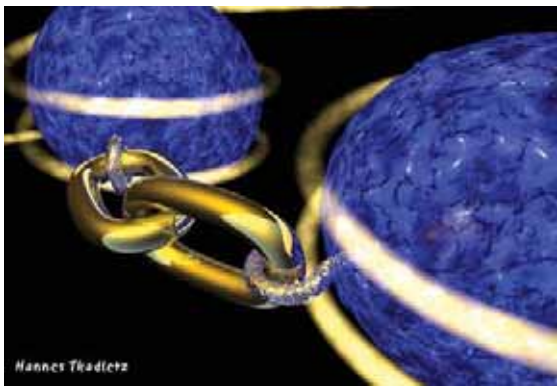

Figure 1: Artistic view for the establishment of heterochromatin by the Suv39h HMTases. Nucleosomes (blue spheres) are cross-linked by the heterochromatin-associated HP1 proteins (golden chains). The affinity of HP1 for heterochromatin is generated by Suv39h-dependent H3-K9 methylation (metal hook).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

the inactive state. Disruption of Suv39h HMTase activities abolishes staining at constitutive heterochromatin but reveals persistent H3-K9 methylation of the inactive X chromosome which, however, fails to localise the heterochromatin-associated HP1 proteins. These data reveal the existence of a Suv39h-HP1 independent pathway in regulating H3-K9 methylation at facultative heterochromatin that may reinforce the specialised chromatin structure of the inactive X chromosome.

Heterochromatin and genome stability

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

Heterochromatin was first described cytologically more than 70 years ago. Because of its stable appearance in the cell nucleus, it has been proposed to have crucial roles in chromosome segregation and the inheritance of cell type identities. Our results have important implications for basic and applied research on higher-order chromatin biology in mammalian systems, ranging from a function of HMTases in centromere identity, chromatid cohesion and genome stability, to their influence in epigenetic gene regulation, X inactivation, imprinting, cell lineage plasticity and nuclear reprogramming. Thus, although our knowledge of SUV39H1 provides a good entry point into SET domain-containing MTases, plenty of exciting surprises will without a doubt surface from further investigations of histone lysine methylation and its role in higher-order chromatin organisation. The impacts for human biology and disease, including cancer and aging, are far-reaching.

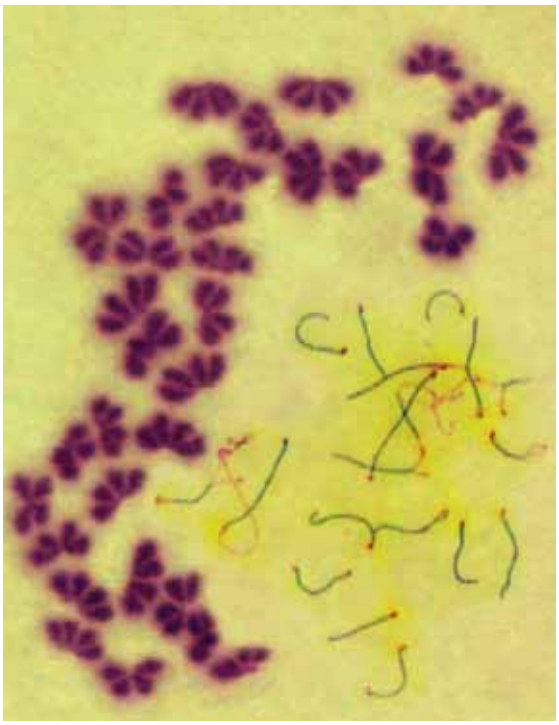

Figure 2: *Suv39h* deficiency impairs genome stability in mitosis and meiosis. Loss of both *Suv39h* genes in the mouse germ line results in genomic instability that is associated with an increased tumor risk and complete spermatogenic failure. Shown are the 'butterfly' chromosomes present in B-cell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

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T CELL TOLERANCE

Tolerance to "self" is a fundamental property of the immune system, and its breakdown can lead to autoimmune diseases such as multiple sclerosis and diabetes. Our lab is interested in the induction and maintenance of T cell tolerance, with particular emphasis on the role of the thymus, a specialized organ that plays an essential role in T cell development. During their functional maturation in the thymus, T cells undergo a series of highly ordered selection processes, which are orchestrated by interactions with different stromal cell types. Our aim is to understand how the biology of thymic stromal cells is related to the generation of a self-tolerant T cell repertoire.

Regulatory T cells

During their development in the thymus, potentially harmful T cells are eliminated from the repertoire (negative selection). In addition, so-called regulatory T cells are induced that can prevent harmful anti-self immune responses in a dominant fashion. Both negative selection and induction of regulatory T cells result from the encounter of self-antigen, and the parameters that influence the choice between either mechanism of tolerance are not understood. One of our goals is to find out how interactions with different thymic stromal cell types and the developmental stage at which an immature T cell encounters self-antigen have an effect on this decision. To do so, we are using T cell receptor and antigen transgenic mice in conjunction with techniques that allow for the generation of genetically chimeric thymi, in order to be able to follow the fate of "self-specific" T cells in various experimental conditions.

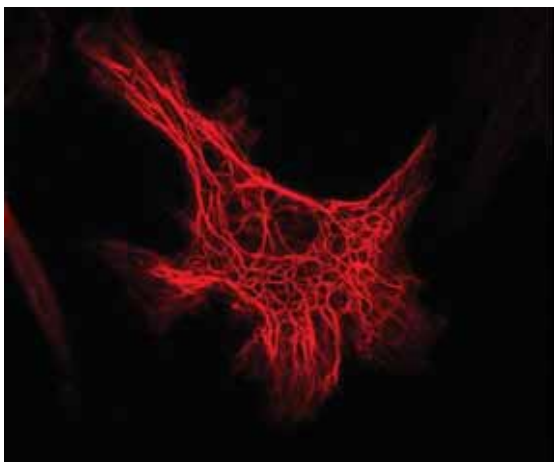

Figure 1: Immunofluorescence of an immortalized thymic epithelial cell, stained for cytokeratin, a typical marker of epithelial cells.

"Promiscuous" expression of self-antigens in the thymus

The extent of self-tolerance imprinted by "central" (intrathymic) mechanisms is limited by the diversity of self-antigens presented in the thymus. For this reason, it was presumed that central mechanisms of tolerance

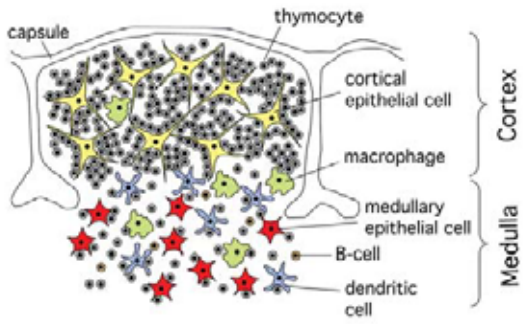

Figure 2: During their maturation in the thymus, developing T cells migrate from the outer cortex to the medulla. On their way, they can make contact with various types of thymic stromal cells. We want to understand how these interaction partners and the differentiation stage at which self-antigen is encountered determine the developmental fate of autoreactive T cells.

predominantly cover abundant or ubiquitous self-antigens, while tolerance to the universe of tissue-specific proteins, whose restricted expression pattern precludes presentation in the thymus, would rely on post-thymic, “peripheral” mechanisms. However, this concept has been called into question by the emerging notion that numerous “tissue-specific” antigens are expressed within the thymus and displayed here for repertoire selection. We found that this „promiscuous“ gene expression is a particular feature of medullary thymic epithelial cells (mTEC). The physiological importance of this phenomenon is underscored by findings that allelic- or strain-dependent variations in the intrathymic expression-levels of certain tissue-antigens correlate with the susceptibility to organ-specific autoimmunity. Along the same lines, we found that absence of central tolerance to a particular epitope of a Central Nervous System (CNS)-autoantigen due to intrathymic expression of a truncated splice-variant influenced the susceptibility to CNS-specific autoimmunity in mice. One focus of our interest is to understand the mechanism(s) underlying the expression of tissue-specific antigens and how this relates to the development of thymus epithelial cells. We are performing a detailed analysis of the phenotype of thymic epithelial cells in normal and mutant mice, in order to compare global gene expression profiles in various subsets of these cells and to elucidate developmental pathways of thymus epithelium. One of our long-term goals is to test whether transplantation of genetically modified thymic epithelial cells can be of therapeutic benefit in autoimmunity.

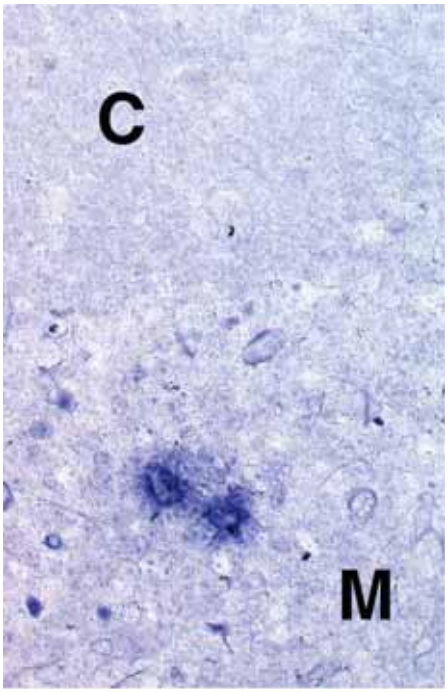

Figure 3: Visualization by *in situ* hybridization of two cells expressing a „liver-specific“ antigen in a medullary region of the thymus (C = cortex; M = medulla)

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ASYMMETRIC CELL DIVISION IN *DROSOPHILA*

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

In *Drosophila*, asymmetric cell divisions play an important role in the development of the nervous system and require function of the conserved protein Numb. 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 *numb* mutants, the daughter cell that normally inherits Numb is transformed into its sister cell, and conversely, overexpression of *numb* causes the opposite cell fate transformation. Thus, Numb acts as a segregating determinant during the development of the *Drosophila* nervous system. How Numb localizes asymmetrically and how it establishes a particular cell fate are the key questions we are trying to answer.

The asymmetric localization of Numb requires a protein called Inscuteable (Figure 1C). Like Numb, Inscuteable localizes asymmetrically, but its localization is found at the opposite side of the cell. In addition, Inscuteable localization begins already before cells enter mitosis. Without Inscuteable, mitotic spindles are misoriented and Numb localizes to the wrong side of the cell cortex. Inscuteable is thought to establish an axis of polarity in interphase that then serves as a reference point for spindle orientation and asymmetric protein localization during mitosis. How does Inscuteable do it? We have used preparative immunoprecipitation to identify Inscuteable binding proteins by mass-spectroscopy and found that a new protein called Pins and a heterotrimeric G-protein α-subunit (Gαi) are associated with Inscuteable *in vivo* (Figure 1D). Like Inscuteable, both proteins localize asymmetrically, and both, Pins and G-protein signaling are required for spindle orientation and asymmetric protein localization during mitosis. Genetic and biochemical experiments have shown that Inscuteable localizes Pins and that Pins activates a heterotrimeric G-protein signaling cascade at one, but not at the other side of the cell. Pins does it in an unconventional way: it dissociates heterotrimeric G-proteins even in the absence of an extracellular signal, and we believe that it acts as a receptor-independent activator of G-protein signaling (Figure 1E). In collaboration with Karl Mechtler at the IMP Protein Chemistry Facility, we are continuing to use mass-spectroscopy to find the downstream components of the signaling cascade and to identify other proteins that mediate the asymmetric localization of Numb during mitosis.

Our biochemical approach is complemented by a large-scale genetic screen for mutants that affect asymmetric

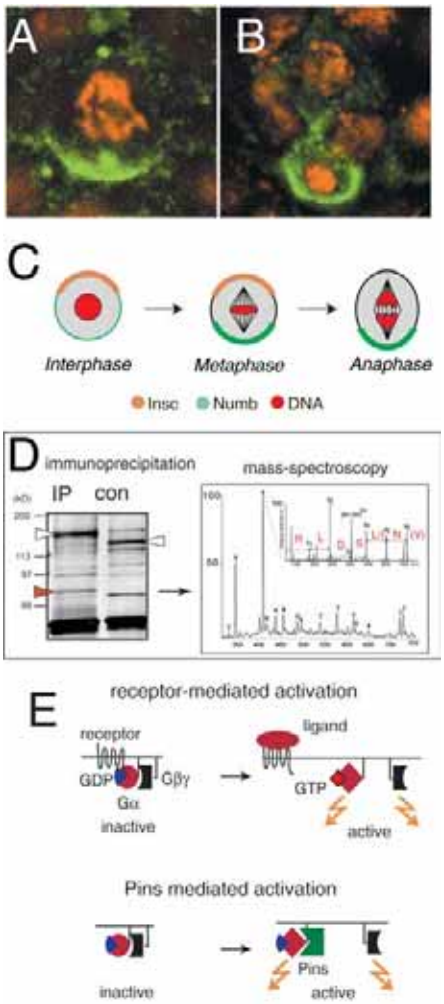

Figure 1: Asymmetric cell division in *Drosophila* neuroblasts.
(A, B) Numb protein (green), DNA (red) and centrosomes (green) in dividing *Drosophila* neuroblasts. Numb localizes asymmetrically in anaphase cells (A) and segregates into one daughter cell in telophase (B). (C) While Numb localization (green) occurs in metaphase and is maintained through anaphase, Inscuteable (orange) localizes asymmetrically to the opposite side in interphase and metaphase, but disappears in anaphase. (D) Identification of Inscuteable binding proteins by preparative immunoprecipitation and mass-spectroscopy. (E) Top: Conventional activation of G-proteins. Ligand binding to the receptor causes GDP/GTP exchange and release of the βγ-subunit. Bottom: Pins can cause the release of the βγ-subunit without the need for any receptor or ligand suggesting that it triggers a cell-autonomous activation of G-protein signaling.

cell divisions in the development of the external sensory (ES) organs. Defects in Numb function and localization lead to characteristic morphological phenotypes in ES organs because their development relies on a series of asymmetric cell divisions during which Numb acts as the segregating determinant (Figure 2A, B). One of the almost 100 mutants we have identified has a phenotype very similar to *numb*: all asymmetric cell divisions during ES organ development become symmetric (Figure 2 C). However, Numb is present and normally localized in this mutant, indicating that the affected gene acts somewhere downstream of *numb* in establishing a particular cell fate. The mutation affects α-Adaptin, a protein involved in receptor-mediated endocytosis. We could show that Numb binds to α-Adaptin and that this interaction leads to the asymmetric localization of α-Adaptin and its preferential segregation into one of the two daughter cells. Thus, the function of Numb is to polarize components of the endocytic machinery. One of the targets of Numb/α-Adaptin mediated endocytosis is the receptor Notch and we are currently investigating how Numb mediated endocytosis affects this important signal transduction pathway.

Asymmetric cell division is crucial for the development of all multicellular organisms. Recent experiments have demonstrated that vertebrate neural stem cells can asymmetrically segregate proteins related to Numb. Therefore, we believe that the mechanisms underlying asymmetric cell division in *Drosophila* will ultimately be applicable to higher organisms, including humans.

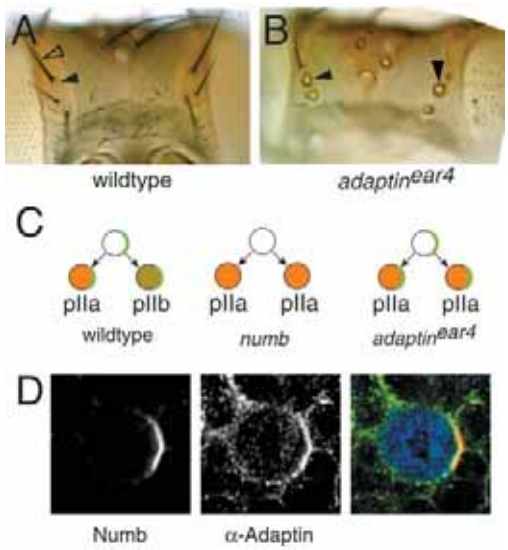

Figure 2: (A) Wildtype *Drosophila* head. Each external sensory organ has one hair (open arrowhead) and one socket (filled arrowhead). (B) In *adaptin*^{ear4} mutants, external sensory organs are abnormal: Hair are missing and replaced by extra socket cells (arrowheads). (C) Like in numb mutants, asymmetric cell divisions become symmetric in *adaptin*^{ear4} mutants even though Numb (green) is present and normally localized. (D) Asymmetric localization of Numb and α-Adaptin. Right: Overlay.
Green: α-Adaptin, red: Numb, blue: Asense (marker for ES organ cells).

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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. Already in 1879, Flemming had noticed that "the impetus causing nuclear threads to split longitudinally acts simultaneously on all of them". What holds sister chromatids together after chromosome replication? What is Flemming's "impetus" that triggers loss of cohesion holding sisters together? How do cells ensure that sister kinetochores attach to microtubules with opposite polarity and that sister separation never occurs before all pairs of sister chromatids have been aligned on the metaphase plate? Such questions are equally pertinent to mitosis and to meiosis during which loss of sister chromatid cohesion between chromosome arms and centromeres must take place at different times.

Genetic and biochemical studies on the budding yeast *Saccharomyces cerevisiae* have identified a multi-subunit complex called cohesin to be essential for holding sister chromatids together during mitosis from the time of DNA replication until the onset of anaphase. Cohesin ensures that sister chromatids attach to microtubules with opposite orientations (known as bi-orientation), which is a precondition for their traction towards opposite poles of the cell. Once chromosomes have bi-oriented, cohesin resists the tendency for sister chromatids to be split apart by microtubules until a cysteine protease called separase cleaves cohesin's Scc1p subunit, triggering the movement of sisters to opposite poles.

How does cohesin bind to chromosomes? How does it hold sister DNA molecules together? How does cleavage of Scc1 break the linkage between sisters? To address these questions, we have investigated cohesin's assembly from its four subunits (Smc1, Smc3, Scc1, and Scc3). The crystal structure of a bacterially expressed SMC "hinge" region along with EM studies and biochemical experiments have shown that cohesin's Smc protomers fold up individually into rod-shaped molecules. A 45 nm intra-molecular coiled coil separates a dimerization region from an ATPase "head" domain. Smc1 and Smc3 bind to each other *via* heterotypic interactions between their dimerization domains to form a V-shaped heterodimer. When co-expressed in insect cells, the two heads of this Smc1/3 dimer are connected by different ends of the cleavable Scc1 subunit, suggesting that cohesin might form a large proteinaceous ring. This raises a possibility that cohesin ring literally embraces sister DNA molecules, and by doing so holds sisters together (Figure 1). We are currently investigating whether cohesin, bound to

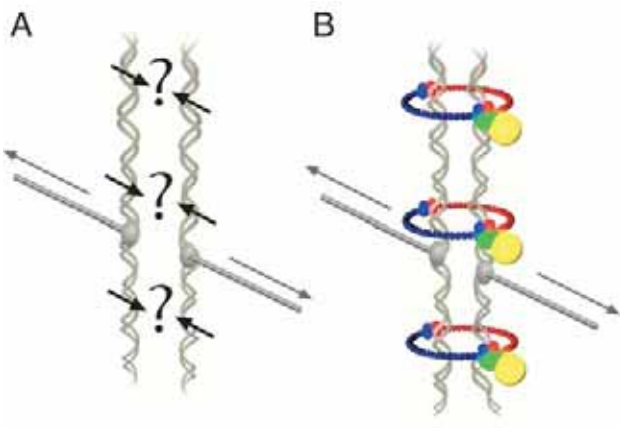

Figure 1: (A) How sister chromatids are held together has long been elusive. (B) Our current model suggests that the cohesin complex connects sister chromatids by forming a ring around them.

chromatids and performing cohesion, forms such rings *in vivo*. Indeed, as predicted by this model, we find that the N- and C-terminal Scc1 fragments produced by separase cleavage at the onset of anaphase are attached to the same Smc1/3 heterodimer. Currently, we are trying to test further predictions of our "DNA embrace" model: first, whether cleavage of the cohesin ring at any point destroys cohesin and causes it to dissociate from chromosomes; second, whether cohesin becomes released from chromosomes by DNA cleavage, and third, whether cohesin's interaction with DNA involves opening of the ring at some point. We are also investigating the role of Scc2/Scc4 complex in loading cohesin onto chromosomes and the role of ATP binding and hydrolysis by Smc1 and Smc3.

Loss of sister chromatid cohesion along chromosome arms is essential for chromosome segregation during meiosis I. Meanwhile; cohesion between sister centromeres persists so that it can later be used to align sisters on the meiosis II metaphase plate. The different timing of sister chromatid cohesion loss between chromosome arms and centromeres is therefore a crucial aspect of meiosis. In budding yeast, a second Scc1-like protein called Rec8p is required 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. During the first meiotic division they disappear from chromosome arms but persist around the centromeres until metaphase II. We have recently shown that the first meiotic division is triggered by separase-induced cleavage of Rec8 along chromosome arms (Figure 2) and we are currently investigating how centromere-localized Rec8 is protected from separase until the second meiotic division. In addition, we are exploring whether cleavage of Rec8 is necessary for the first meiotic division in mice.

Finally, our group is also investigating how chromosomes bi-orient during mitosis and how homologous chromosomes co-orient during meiosis I. We have recently identified a "monopolin" complex whose association with kinetochores during meiosis I is responsible for preventing bi-orientation of sister kinetochores (Figure 2).

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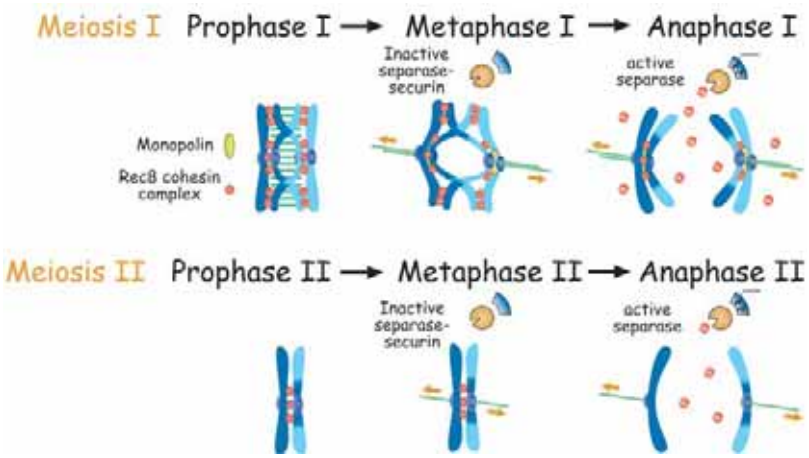

Figure 2: Chromosome segregation during meiosis.

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MOLECULAR MECHANISMS OF VERTEBRATE DEVELOPMENT

The body of all vertebrates is essentially composed of a large number of highly specialized cells. These cells are not randomly scattered but are organized in defined spatial arrangements to give rise to functional units such as organs or body parts. We are using the mouse and chick as model organisms to study the molecular mechanisms that control the development of organized structures from initially very simple groups of cells. Our research focuses on the vertebrate face - a structure that is frequently affected in congenital malformation syndromes in humans.

The vertebrate face develops from buds of tissue, the facial primordia, which surround the primitive mouth (Figure 1). Development of the midfacial region begins with the appearance of the nasal placodes - bilateral ectodermal thickenings at the ventro-lateral sides of the forebrain - that will give rise to the olfactory epithelium. Shortly after the placodes become morphologically apparent the mesenchyme around them starts to grow out to form the nasal processes. Continued outgrowth depends on interactions between the epithelium covering these processes and the underlying mesenchyme. How the areas of mesenchymal outgrowth are established and how the early facial region is patterned is not well understood and is at the focus of our interests.

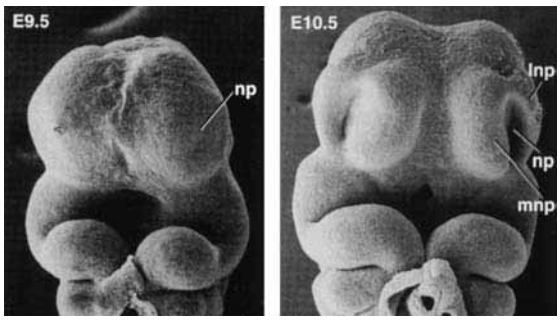

Figure 1: Scanning electron micrographs of the facial region of mouse embryos at E9.5 and E10.5. The nasal placodes (np), thickenings of the facial ectoderm, are the first morphologically distinct structures to form in the prospective midfacial region. By E10.5, the mesenchyme around the placodes has started to grow out to form the medial (mnp) and lateral (lnp) nasal processes and the placodes have now come to lie in shallow depressions, the nasal pits (np, the future nasal cavities), between the nasal processes.

FGF8 function during facial development

FGF8 is a member of the fibroblast growth factor family of signaling molecules. *Fgf8* is widely expressed in the ectoderm covering the midfacial area at early stages of facial development but becomes restricted to a horseshoe shaped domain of expression around the nasal placodes at later stages (Figure 2 A, B). Mouse embryos in which this gene has been inactivated in the facial region develop severe facial defects. Such embryos display midfacial clefts and most derivatives of the first branchial arch are severely reduced or absent (Figure 2 C, D). In early mutant facial mesenchyme the amount of cell death is increased and cell proliferation is reduced. Patterning in the remaining tissue is also affected, in particular in the midfacial area at E9.5 as judged by the analysis of the expression of marker genes. In addition to the mesenchymal defects, also

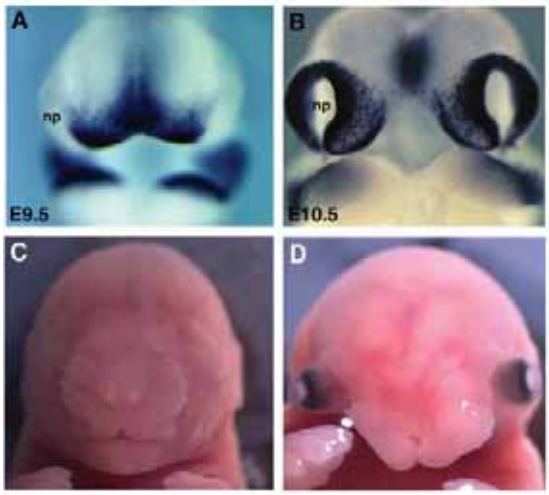

Figure 2: Tissue specific inactivation of *Fgf8* in the facial area results in severe facial defects. Facial expression of *Fgf8* at E9.5 (A) and E10.5 (B). The face of a wildtype (C) and an *Fgf8* mutant embryo (D) at E16.5. Embryos in which *Fgf8* has been inactivated in the facial area develop a midfacial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

the development of the nasal placodes and the surrounding ectoderm is abnormal in *Fgf8* mutant embryos. This includes changes in the expression patterns of ectodermal signaling molecules. Therefore, altered signaling between the mutant ectoderm and the underlying mesenchyme is likely to contribute to the defects observed at later stages.

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

In order to understand how FGF8 controls development of the facial mesenchyme it is important to identify the genes induced or repressed in response to FGF8 signaling. We are using an *in vitro* explant culture system in which facial mesenchyme is cultured in contact with facial ectoderm, in isolation or in contact with polymeric beads soaked in FGF8 protein, to identify such genes (Figure 3).

Using a candidate approach, we have shown that FGF signaling induces the expression of the transcription factors *Pax3*, *Tbx2*, *Erm* and *Pea3* in facial mesenchyme. To systematically screen for FGF inducible genes, we have generated a subtracted cDNA-library from facial mesenchyme cultured in the presence or absence of FGF and have used this library to produce a customized DNA microarray. This micro-array was probed with cDNA derived from mesenchyme cultured with or without FGF. The expression pattern of 200 clones with the strongest differential hybridization was then analyzed by whole mount *in situ* hybridization and inducibility by FGF8 was confirmed. Through this screen we have identified more than 50 genes that are induced in the facial mesenchyme in response to FGF signaling and we have begun to characterize some of them. We believe that this analysis will ultimately help to understand the function of FGF signaling during development at the molecular level.

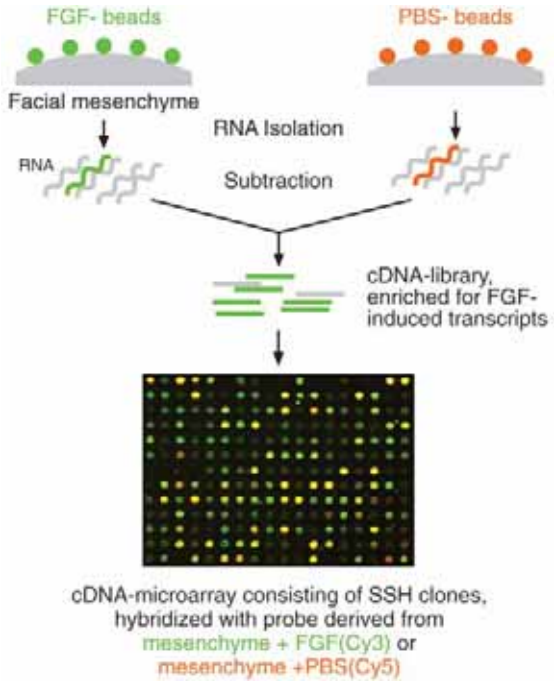

Figure 3: Identification of FGF-inducible genes. Facial mesenchyme was cultured *in vitro* with FGF or PBS soaked beads. RNA was isolated from these explants and used to generate a subtracted (SSH) cDNA-library, enriched for FGF inducible clones. The inserts of 4400 clones from this library were then arrayed on glass slides. The resulting microarray was hybridized with probe derived from RNA isolated from facial mesenchyme cultured with FGF or PBS soaked beads, labeled with a green or red fluorescent dye (Cy3 or Cy5), respectively.

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REGULATION OF MITOSIS IN VERTEBRATE CELLS

The propagation of genetic information during cell proliferation depends on the accurate replication and subsequent segregation of chromosomal DNA. Both of these events are controlled by ubiquitin-dependent proteolysis, a regulatory mechanism that is ideally suited to generate directionality in the cell cycle due to the irreversible nature of protein degradation. We are studying how proteolysis - mediated by the ubiquitin ligase APC (anaphase-promoting complex) and the protease separase - controls 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 (Figure 1). Defects in sister chromatid separation can cause aneuploidy and may contribute to human diseases such as congenital trisomies and cancer.

In eukaryotes from yeast to man sister chromatid separation is initiated by activation of a multi-subunit ubiquitin-protein ligase, called the anaphase-promoting complex (APC) or cyclosome. We have 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 initiate anaphase by activating separase, a protease distantly related to caspases. We are using human cells and eggs of the frog *Xenopus* to address the following topics:

Regulation of sister chromatid separation in vertebrate cells

In yeast the separation of sister chromatids depends on the cleavage of the chromosomal cohesin complex

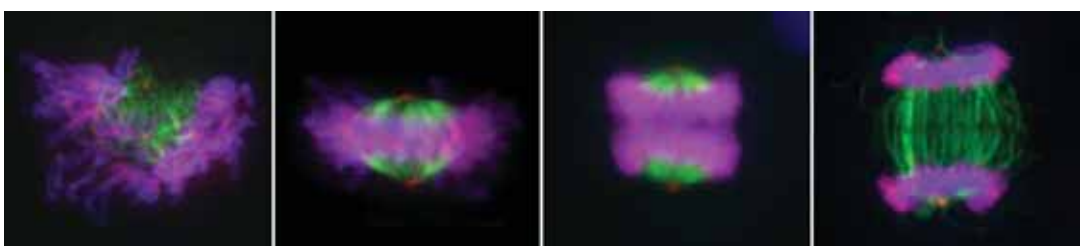

Figure 1: Different stages of chromosome segregation in mitosis. From left to right: cells in prophase, metaphase, anaphase and telophase. DNA (blue), the axes of sister chromatids stained with topoisomerase II antibodies (red), the mitotic spindle stained with tubulin antibodies (green). Courtesy of J.-F. Giménez-Abián.

by separase at the onset of anaphase. We have found that in vertebrates cohesin begins to dissociate from chromosome arms in prophase and from centromeres at the onset of anaphase; the latter event coincides with cleavage of cohesin by separase and is essential for anaphase. Cohesin dissociation in prophase depends on Polo-like kinase (Plk1) but not on separase and is mediated at least in part by cohesin phosphorylation (Figure 2).

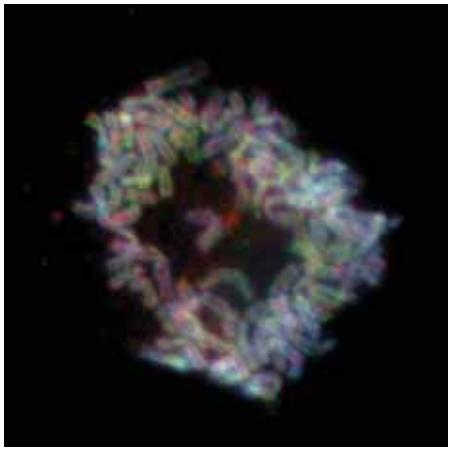

Figure 2: Plk1 is required for complete dissociation of cohesin from chromosome arms. Chromosomes from a cell in which Plk1 expression has been suppressed by RNAi are stained with condensin (green) and cohesin (red) antibodies; DNA (blue). Cohesin is present between sister chromatid arms, whereas in cells containing Plk1 condensed chromosomes only contain cohesin at centromeres (Sumara et al., Mol Cell 9, 515-525, 2002). Courtesy of J.-F. Giménez-Abián and I. Sumara.

To understand the function of cohesin dissociation in prophase we will map and mutate cohesin phosphorylation sites and examine if this yields "non-dissociatable" mutants, and if these mutations affect chromatid condensation and separation *in vivo*.

Function and regulation of human APC and separase

Separase is activated by ubiquitin- and APC-dependent proteolysis of its inhibitor securin. To understand at the molecular level how APC ubiquitinates substrates and how its activity is regulated we are dissecting human APC biochemically and structurally. By analyzing subcomplexes of the APC we hope to obtain insight into the function of individual APC subunits, and by cryo electron microscopy of antibody labeled complexes we aim to identify the relative position of these subunits within the APC. We are also using biochemical and structural approaches to analyze separase. We have shown that securin binding inhibits separase by blocking access of substrates to its active site, and that securin is also required for the proper activation of separase. We have further discovered that securin degradation allows autocatalytic cleavage of separase into a mature form that then cleaves cohesin complexes.

Analysis of mitosis through chemical biology

In collaboration with Boehringer Ingelheim we have identified the small molecule Hesperadin as an inhibitor of chromosome alignment and segregation. We have recently discovered that Hesperadin acts by inhibiting the function of the mitotic kinase Aurora-B. Using this inhibitor as a tool, we have demonstrated that Aurora-B is required for regulation of kinetochore-microtubule interactions and for the proper function of the spindle assembly checkpoint (Figure 3). In the future, we will also use Hesperadin to analyze the function of Aurora-B in other mitotic processes and to identify Aurora-B substrates.

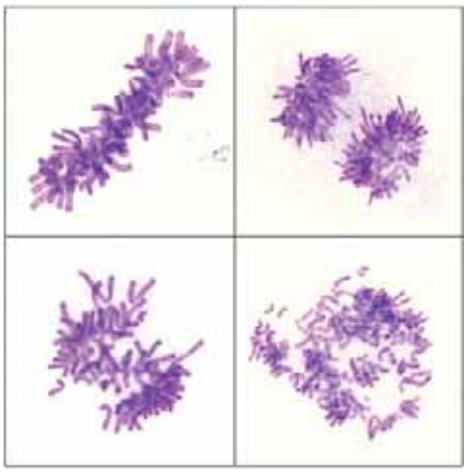

Figure 3: Inhibition of Aurora-B function in human cells causes defects in sister chromatid resolution, and in chromosome congression and segregation. Top: chromosomes from untreated cells in metaphase (left) and anaphase (right). Bottom: chromosomes from cells treated with Hesperadin (Giemsa staining). The resolution of sister chromatids and the congression of chromosomes to a metaphase plate are impaired (lower left). Sister chromatids can nevertheless be separated from each other but fail to be transported towards opposite poles in anaphase (lower right). Courtesy of S. Hauf.

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GENE FUNCTION IN MAMMALIAN DEVELOPMENT AND ONCOGENESIS

The major focus of our group is to unravel gene functions in normal and pathological development, using mouse as a model organism. Specifically, the functions of AP-1 protein family members, e.g. Fos and Jun, are studied in regulation of cell proliferation, differentiation and cell death in organs such as bone, liver, heart, and skin, and in hematopoietic and neuronal development. In addition, specific functions of VEGF, Flk-1 and EGF-receptor are analysed in bone, epithelial and endothelial cells.

Role of Fos and Fra proteins in bone cell differentiation

Fos proteins are key regulators of bone development. Transgenic mice overexpressing c-*fos* develop osteoblastic bone tumours, whereas mice lacking c-*fos* are osteopetrotic and lack osteoclasts (Figure 1). The Fos-related protein Fra-1, itself a c-Fos target gene, is essential for mouse development, whereas transgenic mice overexpressing Fra-1 develop osteosclerosis (Figure 2). Interestingly, gene replacement of c-*fos* by *fra*-1 revealed functional equivalence of these two proteins. To better understand how c-Fos and Fra-1 control osteoblast and osteoclast differentiation, we generated conditional alleles of *c-fos* and *fra-1*. The embryonic lethality of the *fra-1* knock-out mice was rescued by a conditional allele of *fra-1* in *cre*M mouse strain, however the mutant mice developed osteopenia (Figure 2). The conditional allele of c-*fos* was also used to monitor expression of c-Fos during development as well as to study c-Fos function in the CNS (Figure 1).

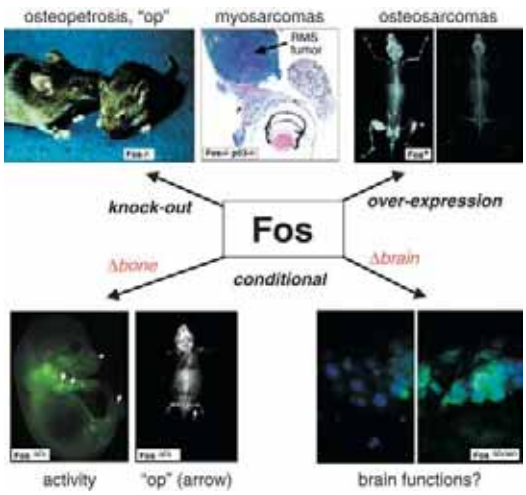

Figure 1: Functional analysis of Fos in bone and CNS development, and in cancer.

Jun/AP-1 function in cell proliferation, differentiation and apoptosis

The functions of the Jun family members are being analysed by conditional mutagenesis, knock-in strategies and transgenic rescue experiments. Specific deletion of *jun* in the liver of adult mice revealed that Jun is dispensable for postnatal liver function, but essential for liver regeneration. Moreover, Jun is required as a survival factor during liver tumour development. Deletion of *jun* in the skin did not affect proliferation of keratinocytes in adult mice, but caused

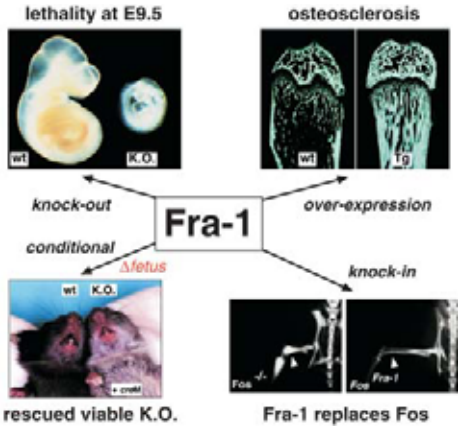

Figure 2: Functional analysis of Fra-1.

an eye closure defect during embryonic development, and skin tumour development. Chondrocyte-specific inactivation resulted in severe scoliosis caused by abnormal intervertebral disc formation suggesting that Jun is a novel regulator of sklerotomal differentiation (Figure 3).

An important mechanism regulating Jun activity is its phosphorylation at serine 63 and 73 by Jun amino-terminal kinases (JNKs). Null mutations in *Jnk1* and *Jnk2* genes (collaboration with M. Karin, UCSD), as well as a *jun* allele mutated in the JNK phosphoacceptor sites (*JunAA*), were generated. *Jnk1*-/-, *Jnk2*-/- and *JunAA* mice are healthy and fertile, however the absence of *Jnk1* and Jun-N-terminal phosphorylation (JNP) results in growth retardation and in fibroblast proliferation defects. *Jnk1*-/- *Jnk2*-/- double mutants develop brain defects, and *Jnk2*-/- and *JunAA* thymocytes are resistant to CD3-induced apoptosis. Moreover, Jnk1 and Jun phosphorylation are required for osteoclast differentiation. Therefore, JNK signalling

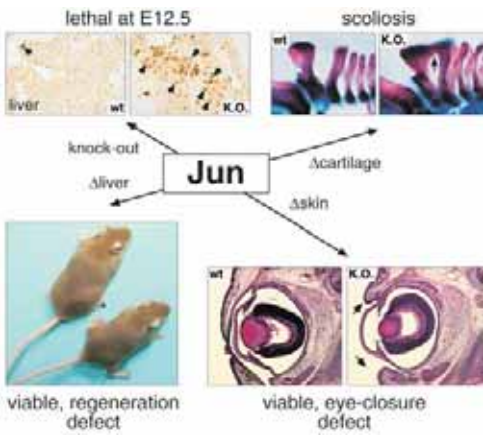

Figure 3: Functional analysis of Jun in development and disease.

and JNP differentially regulate cell proliferation, differentiation and apoptosis in diverse biological processes.

JunB and Fos as tumour suppressors

JunB is a transcriptional activator of the cyclin-dependent kinase inhibitor p16/INK4a and functions as a negative regulator of cell proliferation. Using different *in vivo* approaches including conditional inactivation we have shown that the absence of JunB expression in the myeloid lineage results in a transplantable myeloproliferative disease, which eventually progresses to blast crisis resembling human chronic myeloid leukemia. Thus, JunB was identified as a key transcriptional regulator of myelopoiesis, which controls the number of granulocyte progenitors through inhibition of proliferation and promotion of apoptosis.

When the *fos*-/- osteopetrotic mice were crossed into the p53 background, double mutant mice developed rhabdomyosarcomas (Figure 1). Re-expressing Fos in the double mutant muscle tumour-derived cell lines induced apoptosis revealing a novel, totally unexpected function of the proto-oncogene Fos as a potential tumour suppressor in the muscle lineage.

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

The VEGF/Flk-1 signalling system is essential for the development of endothelial and hematopoietic cells. To test Flk-1's role in adult mice and in tumour angiogenesis a conditional allele of Flk-1 was generated. In addition, a conditional allele of VEGF was used to analyse the functional importance of VEGF-A in developing chondrogenic tissues and to study the role of VEGF-A in skin biology (collaboration with Erwin Tschachler, General Hospital, Univ. Vienna). Finally, conditional and mutated EGF receptor alleles are employed to study the role of EGF-R in skin tumour development (collaboration with Maria Sibilia, Dept. of Dermatology, Univ. Vienna).

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MAMMALIAN X-CHROMOSOME INACTIVATION

For successful development the information encoded by the genome needs to be precisely regulated. During differentiation each individual cell uses an ever-changing repertoire of epigenetic mechanisms to achieve proper regulation of gene expression. Our research focuses on the regulated formation of heterochromatin during the process of X inactivation.

In mammals X-inactivation is required to compensate for the dosage difference of X-linked genes between XY males and XX females. Thereby one of the two X chromosomes in female cells is selected in a random manner and converted into a heterochromatic and transcriptionally inactive chromosome, the inactive X (Xi). Initiation of X inactivation occurs early in development, when cellular differentiation has not yet progressed, and begins with the expression of the *Xist* gene. The mammalian *Xist* gene produces a long, spliced and poly-adenylated non-coding RNA that is uniquely distributed in the nucleus. *Xist* RNA spreads in *cis* from its site of transcription over the entire X-chromosome and mediates the transcriptional silencing of the Xi.

To study the function of *Xist* we have previously developed an inducible *Xist* expression system in mouse ES cells. *Xist* RNA produced from a tetracycline inducible cDNA transgene associated in *cis* with, spread over autosomal chromatin and triggered initiation of chromosome-wide silencing in undifferentiated ES cells (Figure 1). In undifferentiated ES cells silencing is reversible, *Xist*-dependent and independent of chromosomal modifications known to correlate with the inactive X at later stages of cell differentiation. However, upon ES cell differentiation transgenic *Xist* triggered chromosomal modification characteristic of the inactive X in somatic cells. We are using the inducible *Xist* expression system in mouse ES cells to study the initiation phase of *Xist* mediated silencing.

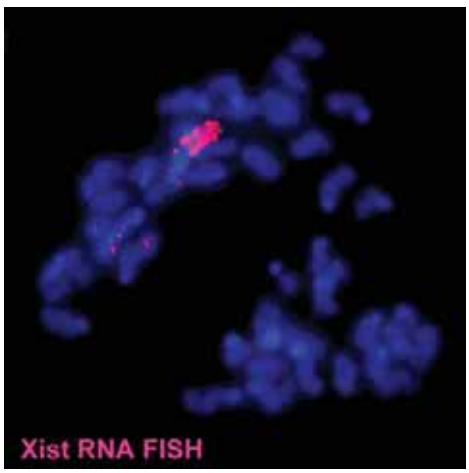

Figure 1: *Xist* RNA association with chromatin revealed by RNA FISH. Mouse metaphase spreads were prepared from ES cells expressing *Xist* from a transgene on chromosome 11 and hybridized with a fluorescent probe detecting *Xist* RNA (red). DNA was counter stained with DAPI (blue).

Identification of the sequences of *Xist* RNA required for localization and silencing

To characterize which *Xist* sequences are required for RNA localization and silencing we have performed a

deletion analysis of the mouse *Xist* RNA. Using a Cre recombinase based transgene homing strategy we have integrated a panel of *Xist* cDNA constructs bearing defined mutations into the Hprt locus on the X chromosome in male ES cells, and characterized the localization patterns of the resulting RNAs and their ability to cause chromosomal silencing. Our results show that chromosomal association of the RNA and transcriptional repression can be separated by specific mutations (Figure 2). Silencing is dependent on the *Xist*

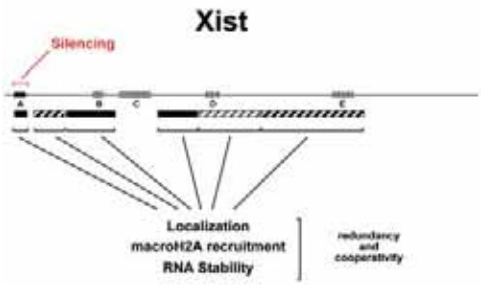

Figure 2: Chromosomal localization and silencing are mediated by different sequences of *Xist*.

repeat A, a conserved repeat sequence located on the 5-prime end of *Xist*. Repeat A consists of seven perfect and one imperfect repetitions of a sequence that can be folded in two stem loops (Figure 3), whereby each copy of the motif is separated by a variable spacer region. Deletion of the repeat A results in an RNA that still associates with chromatin, but does not affect transcriptional repression. Localization of *Xist* RNA to chromatin is a prerequisite for silencing and is mediated by functionally redundant sequences that act cooperatively and are dispersed throughout *Xist*, but share no homology or common sequence motifs.

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

Xist mediated silencing in mice

Initiation of *Xist* mediated silencing is restricted to a stage where cellular differentiation has not yet progressed. Conversely, in differentiated somatic cells *Xist* expression in general does no longer lead to silencing despite the ability of *Xist* RNA to localize to chromatin. We have generated an inducible *Xist* allele to study the initiation of silencing in mouse development. We are especially focusing our studies on the germline stem cells, tumor cells and somatic stem cells. We expect to be able to epigenetically classify cells in the developing mouse as *Xist* responsive or non-responsive and thereby visualize this epigenetic transition *in vivo*.

Functional studies of X inactivation in mice and ES cells should provide insight into the epigenetic regulation of gene expression in mammals. It appears that *Xist* mediated silencing is a paradigm for a powerful epigenetic system that is capable of hetero-chromatinising an entire chromosome and determines its specific nuclear localization. It is expected that similar interactions underlie the regulation of other genes - however, with less dramatic consequences.

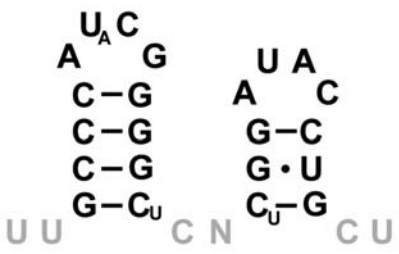

Figure 3: Secondary structure predicted for *Xist* repeat A. The motif shown is repeated seven and a half times, whereby each copy is separated by a variable spacer region.

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SERVICE DEPARTMENT

The Service Department offers a variety of high quality and rapid services to the IMP scientists. The majority of our effort involves DNA sequencing and preparation of various media and solutions.

Our Media Kitchen staff prepares substantial quantities of reagent quality solutions and media for cell culture, flies (approximately 300'000 bottles and tubes per year) and worms. We also prepare many selected reagents such as DNA molecular weight markers, enzymes, a variety of transformation-competent E.coli *strains, and maintain a stock of cloning vectors, primers and other cloning reagents.*

Oligonucleotide Synthesis

The oligoproduction is now outsourced, however we still produce a few oligos for urgent needs.

Production of antibodies

The following services are provided by the Service Department: production of monoclonal antibodies in hybridomas in collaboration with IMP group members, production of polyclonal antibodies by immunization of mice in our animal house facilities, and organization of the antibody production in rabbits with an outside company.

Sequencing and DNA isolation

With the two ABI 3100 Genetic Analyzer capillary sequencers we have sequenced approximately 33'000 samples in the first 9 months of this year. This is a slight increase as compared to 2001. The average read - length on the 3100 Genetic Analyzers equipped with the 80 cm capillaries is 700-900 bases for standard DNA samples. We are saving both, time and money, by using an optimized and fast clean-up protocol with small Sephadex G50 superfine columns on 96-well microtiter plate format and by reducing the amount of sequencing reagents for plasmid DNA and PCR preparations.

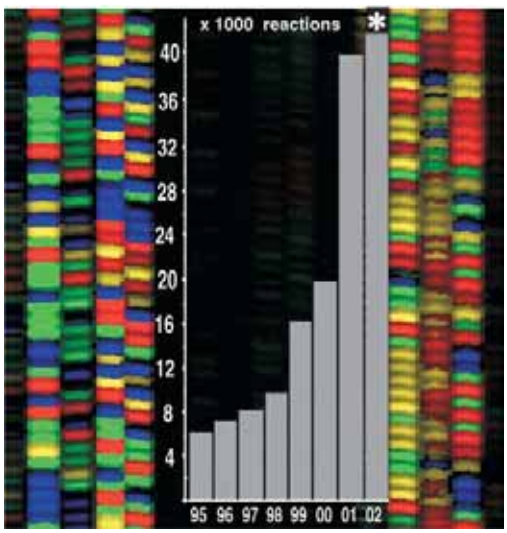

Figure: A sequencing run on an ABI 377 PRISM and number of reactions analyzed on ABI 377 (1995 - 2001) and on ABI 3100 (2001 - 2002) done with dye deoxy terminators (v3.0 since 2001) in the years 1994 to 2002 (scale 0 to 42'000).
*calculated from January 2002 to September 2002 data

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[1]*joint project with H. Beug*

BIOOPTICS DEPARTMENT

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

Flow cytometry

Over the past year we have faced an increasing demand for rare cell sorting, i.e. the isolation of fewer than 0.1% target cells in a cell population. Using automated magnetic cell sorting (AutoMACS), we have established a simple and highly reproducible procedure that improves purity and yield of such rare cell sorts.

Microscopy and image analysis

The last year witnessed the arrival of a spinning disk confocal and a Zeiss LSM510 Meta laser scanning microscopes, both suited for live cell imaging. The spinning disk confocal is specialised for high-resolution 4D-analysis of living cells expressing GFP. The Zeiss LSM510 Meta microscope can perform spectral analysis of multicolour fluorescence, which allows the separation of fluorescence dyes even with highly overlapping emission spectra, e.g. GFP and FITC-signals. Also, technologies like FRET (Fluorescence Resonance Energy Transfer) with high spatial and spectral resolution can now be routinely applied to both, living and fixed samples. To ensure better utilisation of our equipment and facilities, an Intranet-based scheduling and booking system has been implemented.

Microarrays

This year, to improve data analysis, we have almost finished the integration of 13 public databases like LocusLink, Interpro, CDD, or Gene Ontology into our data management system, allowing rapid and comprehensive access to most of the publicly available information. Consequently, it is now possible to quickly assign structural and functional information to clones identified in microarray experiments. We are also implementing a more sophisticated system for the evaluation of microarray data including robust normalisation within and between experiments and application of different clustering algorithms.

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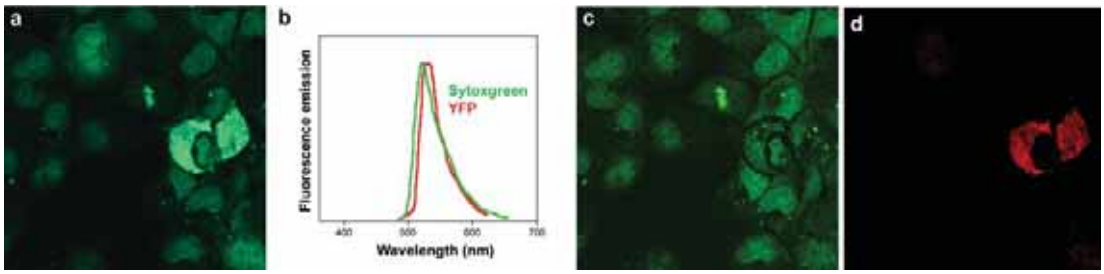

Figure: Cells transfected with YFP were co-stained with Sytox Green and imaged using the Zeiss LSM510 Meta. A spectral analysis was performed and a colour-coded image is shown in A. Although the emission spectra of the two fluochromes highly overlap, as seen in B, a clear separation of the Sytox Green (C) and the YFP (D) signal is apparent.

Animal House

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[1]*since November 2002;* [2]*on maternity leave*

ANIMAL HOUSE

The animal house group provides husbandry of animals and services for the various research groups at the IMP.

Husbandry

The husbandry is divided into three main areas containing the following species: mice, chicken and *Xenopus*. The largest area is the mouse section, where more than 10 000 mice are kept. These comprise breeding colonies, stock and experimental animals including many transgenic and knock-out mouse lines. To provide a constant supply of mice for the various projects, 20 standard strains are routinely bred in-house.

Animal house services

Veterinary services, such as monitoring of the facility´s health-status (sentinel-program etc.), experimental procedures in animals such as collection of blood, implantation of tumor cells and administration of substances by iv, ip or sc injections. All procedures are performed to a high standard under appropriate anaesthetic regimes and in conjunction with the necessary project licenses.

Animal procurement, such as ordering of mice from external breeding companies, organizing and handling of approximately 50 incoming and outgoing mouse-shipments per year.

Administration of regulatory affairs in accordance with the Austrian laboratory animal law, which include record-keeping and updating of laboratory animal statistics, and specific documentation of laboratory animal experiments.

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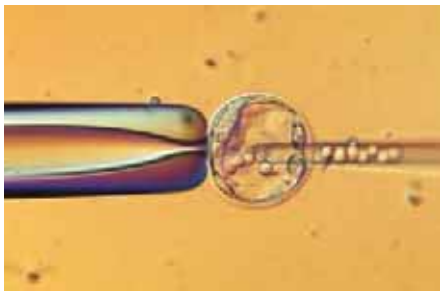
Mouse service department

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 for the transfer of 'clean' embryos into our animal house, the freezing of embryos for the preservation of specified mouse strains and the teaching of basic embryological techniques to the IMP staff. *In vitro* fertilization experiments (IVF) are performed and the mouse strain data base is kept up-to-date. About 30 different ES cell clones and several DNA constructs are being successfully injected per year. The activities of this department are overseen by an Animal User Committee, which meets bimonthly to set priorities and to coordinate the duties. At present, it is chaired by Erwin F. Wagner.



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Jan-Michael PETERS / Scientific Coordinator

Mathias MADALINSKI / Technician

Ines STEINMACHER / Technician

PROTEIN CHEMISTRY FACILITY

The IMP Protein Chemistry Facility performs a large variety of mass spectrometry experiments, including identification of proteins by peptide sequencing and characterization of post-translational modifications such as phosphorylation. In addition we develope new methods to increase sample throughput, sequence coverage and sensitivity in mass spectrometry. Finally, our facility specializes in peptide synthesis and antibody purification.

Shotgun 2D Proteomics technology for the analysis of post-translational modifications in protein complexes

Tandem mass spectrometry (MS/MS) experiments are capable of generating short stretches of sequence information but in most cases only a small fraction of all generated peptides can be recovered and analyzed. To overcome this problem we have developed the following "shotgun" approach. First, protein complexes are purified by immuno-precipitation and are subsequently digested by different enzymes: one that cleaves in a site-specific manner and one that cleaves non-specifically. Next, the mixture of peptides is separated by nano-HPLC or by the combination of multiple chromatography steps ("multi-dimensional liquid chromatography") and is analyzed by tandem mass spectrometry. By combining the high sensitivity and the resolution of nanoscale multi-dimensional liquid chromatography with the precise structural specificity of MS/MS spectral data we identify the sites and types of modifications in large portions of the sequence of our protein complexes. By using proteases with different cleavage specificities we generate overlapping peptides. The analyses of overlapping peptides increase both the certainty with which modifications can be identified and the likelihood of obtaining peptides, from the "high-quality" MS/MS spectra can be obtained. This approach is particularly important for analyzing the complexity of multi-subunit protein complexes.

Peptide synthesis and antibody purification

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

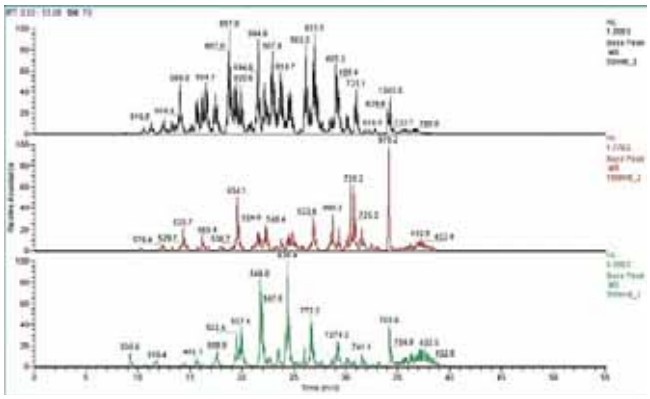

Figure: Shotgun Proteomics. Peptides were trapped on a SCX column normally at pH~3. At this pH value, the peptides are positively charged and the single charge is usually sufficient for the binding. Peptides were eluted from the SCX column with increasing ammonium acetate salt steps (from the top) onto the reversed phase column. Peptides obtained by this separation procedure were sequenced and analyzed using MS and the database search of generated MS-MS spectra.

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(*equal contribution; † equal contribution of senior authors)

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AWARDS AND HONOURS IN 2002

Jan-Michael Peters

Novartis Research Prize for Biology

(Vienna, January 25, 2002)

Jürgen Knoblich

Thomas Jenuwein

Jan-Michael Peters

Elected EMBO members

(Heidelberg, May 8, 2002)

Nicole Firnberg

Sebastian Maurer-Stroh

Christine Zimmer

NAWI-CLUB prize (Friends of the Faculty of Science at the University of Vienna)

(Vienna, June 7, 2002)

Alexandra Schebesta

Science prize 2002 of the government of Lower Austria

(Krems, Donau University, November 3, 2002)

Attila Toth

Amersham Biosciences and Science Prize for Young Scientists 2002

(for his essay: “Cohesin and Monopolin: Two Major Determinants of Chromosome Segregation,” based on his PhD work in Kim Nasmyth’s group)

(Cambridge, UK, November 22, 2002)

Seminar speakers at the IMP in 2002

January

11.01.02 ANDREA MUSACCHIO (EIO, Milan)
FRANCOIS TRONCHE (CNRS, Paris,)
15.01.02 DIRK LANKENAU (Univ. Heidelberg)
18.01.02 MAIKE SANDER (ZMNH, Hamburg)
21.01.02 OLGA MAYANS (Univ. Basel)
31.01.02 DAVID BARFORD (ICR, London)

February

08.02.02 ANDREA VORTKAMP (MPI, Berlin)
14.02.02 FRANS VAN ROY (Gent Univ. Belgium)
28.02.02 OLKE UHLENBECK (Univ. Colorado)

March

04.03.02 LARS GROTEWOLD (Heinrich-Heine Univ. Düsseldorf)
06.03.02 ANDREAS VILLUNGER (WEHI, Melbourne)
11.03.02 ALEXEY GORDADZE (Baylor College of Med., Houston)
12.03.02 CHRISTIAN BOGDAN (Univ. Erlangen-Nürnberg)
15.03.02 COLIN ROBINSON (Warwick Univ.)

April

04.04.02 MATTHIAS WABL (UCSF)
09.04.02 KAREL RIHA (Texas A&M Univ.)
10.04.02 LENA ALEXOPOULOU (Yale Univ.)
10.04.02 CLAUDIO PUNZO (Univ. of Basel)
11.04.02 AUSTIN SMITH (Edinburgh Univ.)
12.04.02 RALPH NEUJAHR (Harvard Med. School)
12.04.02 ROBERT C. GALLO (Univ. of Maryland)
(IMP CANCER LECTURE IN MEMORIAM LAURA STINGL)
16.04.02 WILFRIED ELLMEIER (Univ. of Vienna)
17.04.02 ELAZAR ZELZER (Harvard Med. School)
17.04.02 KARL-PETER HOPFNER (Univ.Munich)
18.04.02 MARINO ZERIAL (MPI, Dresden)
19.04.02 LUDGER KLEIN (Dana-Farber Cancer Inst.)
24.04.02 IGOR BEREZOVSKY (Weizmann Inst., Rehovot, Israel)
25.04.02 MICHAEL SENDTNER (Julius-Maximilians-Univ., Würzburg)
26.04.02 BUZZ BAUM (Ludwig Inst., UCL, London)
30.04.02 ADELHEID CERWENKA (Novartis, Vienna)

May

02.05.02 KATHY WILSON (John Hopkins Univ., Baltimore)
07.05.02 MIA HOROWITZ (The Hebrew Univ., Jerusalem)
07.05.02 MICHAEL AMLING (Univ. School of Med., Hamburg)
08.05.02 JOHN ROSS (Stanford Univ.)
13.05.02 CHI-BIN CHIEN (Univ. of Utah)
14.05.02 PETER ten DIJKE (Netherlands Cancer Inst.)
15.05.02 GERALDINE SEYDOUX (Johns Hopkins Univ. School of Med., Baltimore)
15.05.02 ELISA BOFILL (Univ. of Vienna)
16.05.02 ELIZABETH SMYTHE (Univ. of Dundee)
22.05.02 MARIE-NOELLE PRIOLEAU (Ecole Normale Supérieure, Paris)
28.05.02 IDIT SHACHAR (Weizmann Inst., Rehovot, Israel)
29.05.02 DOUGLAS HANAHAN (UCSF)

June

06.06.02 TRINA SCHROER (John Hopkins Univ.)
10.06.02 STEPHAN GRILL (MPI, Dresden)
11.06.02 JOHN QUACKENBUSH (TIGR, Rockville)
13.06.02 UDO HÄCKER (Dep. of Cell and Mol. Biol., Lund Univ.)
14.06.02 STEPHEN TAYLOR (Manchester Univ.)
20.06.02 PHILIPPE COLLAS (Oslo Univ.)
21.06.02 FRANK GERTLER (Massachusetts Inst.)
27.06.02 CHRISTOF NIEHRS (DKFZ, Heidelberg)

July

04.07.02 JEFF ERRINGTON (Univ. of Oxford)
09.07.02 MARTIN ZENKE (MDC, Berlin)
11.07.02 ALAIN CHEDOTAL (INSERM, Paris)
12.07.02 RUEDIGER KLEIN (MPI, Martinsried)
18.07.02 WALTER BIRCHMEIER (MDC, Berlin)
19.07.02 ANA CUMANO (Inst. Pasteur, Paris)
29.07.02 BARBARA ZANGERL (Cornell Univ., Ithaca)

August

02.08.02 CLARE BAKER (Cambridge Univ.)

September

04.09.02 CHIARA ABRESCIA (Univ. of Naples)
05.09.02 SEAN MUNRO (Univ. of Cambridge)

06.09.02 MIKE FERGUSON (Univ. of Dundee)

13.09.02 TORU HIROTA (Kumamoto, Japan)

13.09.02 JAMES WITOWSKY (Univ. Colorado, Denver)

23.09.02 ANDREAS HOENGER (EMBL, Heidelberg)

25.09.02 MARTIN OFT (DNAX, Palo Alto)

26.09.02 WALTER WITKE (EMBL, Monterotondo)

October

09.10.02 TADATSUGU TANIGUCHI (Univ. of Tokyo)

10.10.02 STANLEY FROEHNER (Univ. of Washington)

17.10.02 ANJANA RAO (Harvard Med. School)

18.10.02 PETER GREENBERG (Univ. of Iowa)

21.10.02 STEVE BURDEN (NYU Med. Center)

25.10.02 HARALD ZUR HAUSEN (DKFZ, Heidelberg)

November

05.11.02 RUDOLF HAMMER (Boehringer Ingelheim, Japan)

07.11.02 RON KOPITO (Stanford Univ.)

20.11.02 ANTONIO LANZAVECCHIA (IRB, Bellinzona)

21.11.02 LESLIE VOSSHALL (Rockefeller Univ.)

22.11.02 MARCOS GONZALEZ (MPI, Dresden)

26.11.02 STEFAN HOENING (Univ. of Göttingen)

28.11.02. MATTHIAS PETER (ISREC, Lausanne)

29.11.02 THORSTEN HEINZEL (Inst. for Biomed. Res., Frankfurt)

December

03.12.02 OLIVER BRUESTLE (Univ. of Bonn)

05.12.02 JOHN WALKER (Cambridge Univ.)

12.12.02 MICHAEL LICHTEN (NCI, Bethesda)

17.12.02 ANNE-KARINA PERL (Cincinnati Children's Hospital)

19.12.02 RICHARD KOLODNER (Ludwig Inst. for Cancer Research, San Diego Branch)

20.12.02 JEAN Y.J. WANG (UCSD Cancer Centre)

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Erwin... near the top of the world

Kim´s surprise birthday party

Kids Christmas party at the IMP

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Annette and Christine at the Lucca Conference

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