RESEARCH INSTITUTE OF MOLECULAR PATHOLOGY

VIENNA BIOCENTER

2006

Boehringer Ingelheim

IMP
Research Institute of Molecular Pathology

CONTENTS

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2006 has been an eventful year for the IMP, and indeed for the entire Campus Vienna Biocenter. At the IMP, we are delighted to have been able to recruit three new junior group leaders: Carrie Cowan, from the Max Planck Institute of Cell Biology and Genetics in Dresden, Simon Rumpel, from Cold Spring Harbor Laboratories, and Stefan Westermann, from the University of California at Berkeley. Additionally, we have launched the IMP Fellows program, offering independent postdoctoral positions to talented young scientists right after their PhD. Peggy Stolt-Bergner is our first fellow, joining after a PhD at Harvard and a short postdoctoral stint at the Max Planck Institute of Biophysics in Frankfurt.

The IMP also continues to invest heavily into new technologies, with an emphasis over the past year on microscopy. Katrin Heinze joined us as an independent staff scientist, and will collaborate with the research groups and Biooptics service in developing new optical systems for 2-photon imaging and fluorescence correlation spectroscopy. Together with IMBA, we have also obtained generous support from the City of Vienna to set up a state-of-the-art electron microscopy facility, headed by Günter Resch, and including cryoelectron microscopy together with the research group of Thomas Marlovits.

The IMP has also changed physically over the past year. The spacious new Academy building has opened up next door, housing IMBA, GMI, and several joint IMP-IMBA service departments. Proteomics, histology, DNA sequencing and the media kitchen have all moved over, and EM has been set up there. We hope the animal house will soon follow. This move has triggered further renovations at the IMP. This includes the wonderful new IMP-IMBA kitchen and cafeteria, which now not only offers a more attractive setting, but also new culinary delights introduced by the enlarged cafeteria team and our consultant, Winni Brugger. The cafeteria is once again the hub of the social and scientific life at the IMP and IMBA, and is proving to be a magnet for scientists from the whole campus. Some of the labs have also been remodelled. The walls came down betw een the labs on the first floor, and the ground floor renovated to provide new space for flies, protein chemistry, electrophysiology, and advanced light microscopy. The technical department has done a heroic job to oversee all this reconstruction, while somehow still managing to maintain normal operations in both buildings. Many thanks to Alex Chlup and his crew!

Our neighbours, too, are changing. On one side of us, IMBA and GMI are now up-and-running in their new building. On the other, the Biocenter of the Universities has now been reorganized as the Max F. Perutz Laboratories (MFPL). Graham Warren from Yale has been appointed as the MFPL's first Scientific Director. We are looking forward to working with Graham and all our colleagues from the MFPL to further strengthen the scientific and social interactions between the IMP and MFPL.

As always, we are extremely grateful for the continued advice from our SAB, and for the generous support from Boehringer Ingelheim and various funding agencies. We were particularly pleased that Boehringer Ingelheim decided this year to hold its international R&D press conference at the IMP, emphasizing its strong commitment to basic, curiosity-driven research. At a time when even governmental agencies are increasingly expecting short-term returns on their investments in basic science, it is deeply satisfying and we hope exemplary that a private company maintains such a visionary, long-term perspective.

We thank Boehringer Inhelheim, the SAB, and all IMP researchers for another outstanding year of science, and all IMP and IMBA staff members for their support, cooperation, and patience during the many upheavels of the past year.

The IMP and Its Surroundings

The Research Institute of Molecular Pathology (IMP) is a basic biomedical research center in Vienna, the capital of Austria. Its major sponsor is Boehringer Ingelheim, a globally operating pharmaceutical company with its headquarters in Germany. The knowledge created at the IMP is at the disposal of Boehringer Ingelheim for the development of innovative diagnostic and therapeutic concepts.

The Campus Vienna Biocenter

Opened in 1988 close to the city center, the IMP triggered the development of its neighbourhood into a teeming biotechnology hub. Today's "Campus Vienna Biocenter" (VBC) is also home to the Max F. Perutz Laboratories (University and Medical University of Vienna), the Institute of Molecular Biotechnology IMBA and the Gregor Mendel Institute GMI (Austrian Academy of Sciences), a University of Applied Sciences, several biotech companies, a PR agency, a non-profit scientific society and the Vienna Open Lab.

Close ties have been knit between the IMP and IMBA: the institutes are not only linked physically by an attractive skywalk, but cooperate closely on all levels and share large parts of their infrastructure. The collaboration is known under the name "IMP-IMBA Research Center".

More than 1000 people from 40 different nations currently work at the Campus VBC. Campus 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 lectures, are open to all.

Vienna – a City of Many Facets

For those whose interests stretch beyond science, Vienna also has a lot to offer. Home to about 1.6 million people, the city is the political and cultural center of the country and its gateway to the east. Once the heart of the largest European empire, Vienna draws on a rich cultural heritage which is reflected in splendid buildings and unique art collections. But Vienna is a city of many facets. Modern architecture, splendid galleries and stylish shops are as much a part of everyday life as the famous concert halls, the big museums and the nostalgic little streets. As any European capital, Vienna also offers a vibrant nightlife, with a rich selection of restaurants, cozy bars, and trendy clubs.

Apart from Vienna's focus on art and culture, it also has a long-standing tradition in science. Founded in 1365, the University of Vienna is the oldest university in the German-speaking world and the largest in Austria. 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.

And if your brain needs to be refreshed, there is always the call of nature. A day away from the lab may take you skiing to the nearby Alps, windsurfing at Lake Neusiedl or watching rare birds in the Seewinkel. Even within the borders of Vienna, you can enjoy a hike through dense woods, go canoeing in a National Park, climb impressive limestone cliffs or stroll through rolling vineyards. In fact, more than 50% of Vienna's surface area is covered by vegetation.




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 state-of-the-art scientific services. As a member of one of the scientific groups, you will be part of a young, international team, using English as a working language. The unique "flavor" of the IMP lies in its stimulating and focused atmosphere, where science always comes first but social activities are not neglected. Informal interaction is strongly encouraged as a vital prerequisite for intellectual exchange. Not least, our famous cafeteria offers well-deserved breaks away from the lab.

Graduate students join the IMP through the Vienna Biocenter International PhD Program, run jointly with the Max F. Perutz Laboratories, the Institute of Molecular Biotechnology (IMBA) and the Gregor Mendel Institute (GMI). A call for applications goes out twice a year, with contracts typically lasting 3-4 years. At present, more than 50 students are working towards their PhD at our institute.

A new kind of position has been created this year: IMP fellowships offer young scientists the unique possibility of conducting creative and independent research at a very early stage in their career. IMP fellows join the IMP within a year of obtaining their PhD degree, and are given a four-year contract. They are granted their own budget and travel allowance and can take full advantage of the scientific infrastructure of the institute. A mentoring committee monitors progress and ensures independence. IMP fellows will be considered for promotion to a group leader position within the first three years. The IMP´s first fellow started in August: Peggy Stolt-Bergner.

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. A substantial travel budget allows scientists to take part in meetings, conferences and courses. The IMP and IMBA organize a couple of conferences, workshops and scientific meetings every year. Students are successfully organizing their own symposia, backed by a generous budget from the institute. 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 in the first place for the science. We do, however, appreciate your private needs and try to make relocation as smooth as possible. For newcomers, there are several in-house apartments to bridge the time until they have found a place of their own. Our administrative staff is helpful in finding housing and our personnel department will take care of your legal requirements including visas, registration, health insurance and family matters. For parents with young children, two day-care facilities are available in the immediate neighborhood: a municipal kindergarten offers admission from baby-age, and a privately run nursery is also just around the corner. For school-age children, Vienna offers a large range of different types of schooling, from public to private, German- or foreign language-speaking, traditional or with more experimental concepts.

Many of our new employees are accompanied by spouses who are themselves looking for a qualified position in line with their training. The IMP is certainly aware of this fact and can, in some cases, help with securing a job. We also support your efforts to learn German and sponsor language courses run by one of Vienna's best language schools. In addition to caring for the intellect, the IMP features a soccer club and subsidizes regular physical activities for its members.

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

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Oncogenesis: Abnormal Developmental Plasticity?

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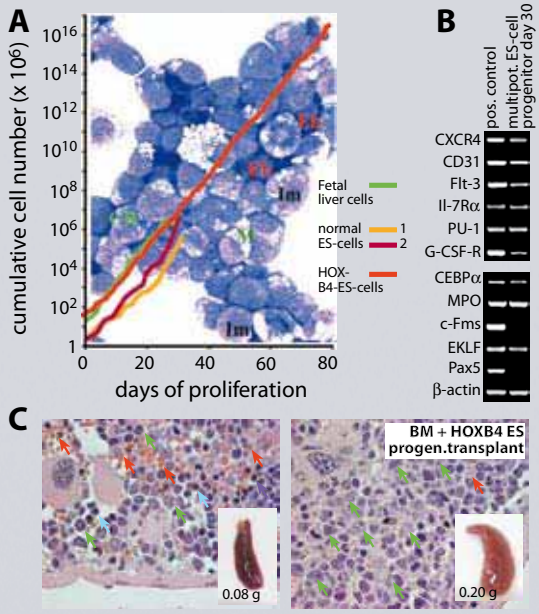

In leukemia and carcinoma development, the cooperation of oncogenic receptors/signal transducers with signal-activated, sometimes mutated transcriptional regulators causes abnormal proliferation, survival and developmental plasticity. Genetically modified mice and novel cell culture models derived thereof are combined to investigate the mechanisms as how such oncogene combinations regulate self-renewal versus differentiation of hematopoietic progenitors and epithelial plasticity/trans-differentiation of carcinoma cells during metastasis.

Mechanisms of Hematopoietic Progenitor Renewal and Leukemogenesis

Primary erythroid progenitors undergo self renewal in vitro through cooperation of the EpoR, c-Kit and glucocorticoid receptor (GR), reflecting a physiological response to stress erythropoiesis. An optimal cell model to analyze progenitor renewal are erythroblasts from murine ES-cell-derived embryoid bodies (ESEPs). These cells show in vivo-like responses to renewal and differentiation factors, are diploid and genetically stable, have an unlimited lifespan in culture and allow analysis of erythropoiesis in early embryonic lethal mouse mutants (e.g. Flk-1-/- mice). Flk-1-/- ESEPs fail to undergo long-term erythro-blast renewal due to enhanced differentiation, a defect rescued by FGF-1. Clonal multipotent progenitors can likewise be expanded from ES-cells, particularly if expressing exogenous HoxB4 (Figure 1A, B). In mice, such cells cause a myeloproliferative disease (Figure 1C). In these models, we will analyze the function of STAT-1, -3, -5, the GR and cytokine-driven upstream signaling pathways (e.g. Jak2), employing or newly generating respective, homozygously defective ES cells. These models will also serve to study human leukemia oncogenes, e.g. mutated receptors/signal transducers (Flt3/c-Kit/Jak2) cooperating with transcription factors/chromatin regulators (ca-STAT5, RAR/PML, AML-ETO). Polysome-bound mRNA expression profiling of these models will help to identify new key genes regulating the balance between self-renewal and differentiation (with collaborators).

ILEI, a Novel Secreted Protein Essential for Epithelial Plasticity and Metastasis

Epithelial/mesenchymal transition (EMT) and metastasis of polarized mamary epithelial cells (EpH4) – caused by oncogenic Ras (EpRas) plus TGFβ – mirrors late stage cancer progression and requires a hyper-active Ras-MapK pathway. Polysome-bound mRNA expression profiling identified ≈30 EMT-specific genes, many of which contribute to EMT and metastasis (e.g. ΔEF-1, annexin-A1, NFκB and PDGF-receptor signaling; Figure 2A). The most exiting EMT-specific gene identified was the interleukin-like EMT inducer (ILEI), a translationally regulated, secreted protein lacking sequence homology to known genes. Stably over-expressed

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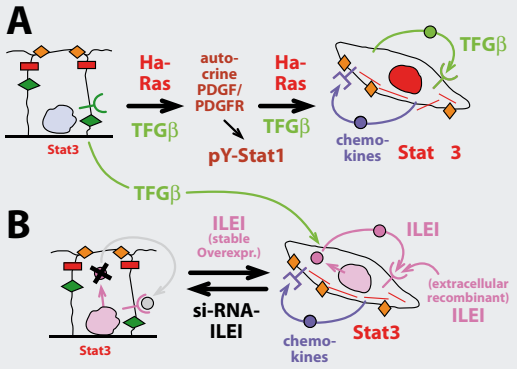

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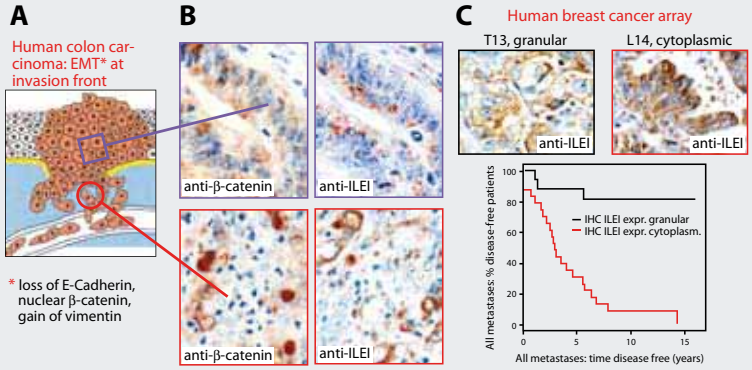

Figure 1: *HOXB4 promotes long-term renewal of ES-cell-derived multipotent progenitors. (A). Multipotent progenitors from ES-cell-derived embryoid bodies (day 8) expressing HOX-B4 (red) show long-term expansion in multipotent medium mix (SCM), while fetal liver cells (green) and uninfected ES-derived cells (yellow, brown) show medium-term expansion. Cytospins show immature cells (Im), erythroid (Eb) and various myeloid progenitors (M, GR). (B). HOX-B4 multipotent progenitor clones express markers of erythroid, myeloid and lymphoid lineages (RT-PCR). (C). Lethally irradiated NOD/SCID mice transplanted with HOX-B4-multipotent ES-derived cells plus wt bone marrow develop a myeloproliferative disease in the spleen, showing excessive production of myeloid cells (green arrows) at the expense of erythroid and lymphoid progenitors (red and blue arrows).*

Figure 2: *Diagram illustrating molecular events during Ras/TGFβ- and ILEI-dependent EMT. (A). Expression of oncogenic Ha-Ras plus exposure to TGFβ induces EMT in EpH4 cells, involving upregulation of an autocrine PDGF/PDGF-receptor loop, as well as induction of chemokine/receptor autocrine loops that contribute to EMT as shown by receptor antagonists. These autocrinely acting chemokines may be responsible for upregulation of STAT3 during EMT, the latter beeing stabilized by a TGFβ-autocrine loop. (B). Stable expression of exogenous ILEI, addition of recombinant ILEI or TGFβ-induced upregulation of endogenous ILEI cause EMT in EpH4 cells and derivatives. Stable ILEI expression also induces autocrine chemokines important for EMT and STAT3 upregulation. All these processes are inhibited or reversed by RNAi-mediated knockdown of ILEI.*

Figure 3: *Delocalization of vesicular LEI correlates with EMT and metastasis in human carcinomas. (A, B). ILEI is expressed in apical vesicles in colon carcinoma primary tumor cells (A, blue square) that express cytoplasmic β-catenin (B) and membrane-localized E cadherin. Tumor cells at the invasion front (A, red circle) that have undergone EMT as shown by nuclear β-catenin (B), loss of E-cadherin and gain of vimentin show strong, cytoplasmic ILEI expression (B). (C). Correlation of vesicular (top left) and cytoplasmic ILEI staining (top right) in a large human breast cancer array with patient histories recorded over 15 years shows that cytoplasmic ILEI expression predicts high metastasis incidence (bottom panel) and bad prognosis (not shown).*

ILEI was sufficient to induce EMT, slow tumor growth and aspects of metastasis in EpH4- and NMuMG cell lines, enhanced by co-expression of Bcl-2. Bioactive, recombinant ILEI purified from transiently transfected mammalian cells also induced EMT. Endogenous ILEI was essential for both EMT and metastasis, as shown by RNAi (Figure 2B). Besides generating cell lines stably producing correctly processed, bioactive ILEI, we will focus on chemokines induced by stably expressed ILEI. These chemokines play a role in Ras/TGFβ- and ILEI-dependent EMT and induce upregulation of nonphosphorylated STAT3 (Figure 2B), apparently inducing cancer-related genes via unknown mechanisms which we want to investigate. ILEI expression is restricted to apical, Golgi-like vesicles in normal epithelia, but shows delocalized cytoplasmic expression in human tumors, correlated with EMT at invasion fronts (Figure 3A, B), metastasis and bad prognosis in human breast cancer (Figure 3B, C). We will thus investigate whether abnormal vesicle transport/secretion of ILEI is essential for human tumor progression. This may involve Annexin-A1, which causes EMT and metastasis when knocked down via RNAi in normal EpH4 cells.

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MEINRAD BUSSLINGER
Stem Cell Commitment in Hematopoiesis

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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 transcription factors such as Pax5 and Notch1 control the commitment of early hematopoietic progenitors to the lymphoid lineages.

B Cell Commitment

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 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 was thus 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. We now identify and characterize the control regions and DNA-binding factors that determine the B-cell-specific expression of *Pax5*.

Molecular Mechanism

At the molecular level, Pax5 fulfils a dual role by activating the expression of B-cell-specific genes and by repressing the transcription of lineage-inappropriate genes. To systematically analyze the transcriptional function of Pax5, we 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). A large part of the 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. The Pax5-dependent repression of these lineage-inappropriate genes is essential for normal homeostasis of hematopoietic development, as ectopic expression of the Pax5-repressed chemokine gene *Ccl3* in B cells results in increased osteoclast formation and bone loss (Figure 3).

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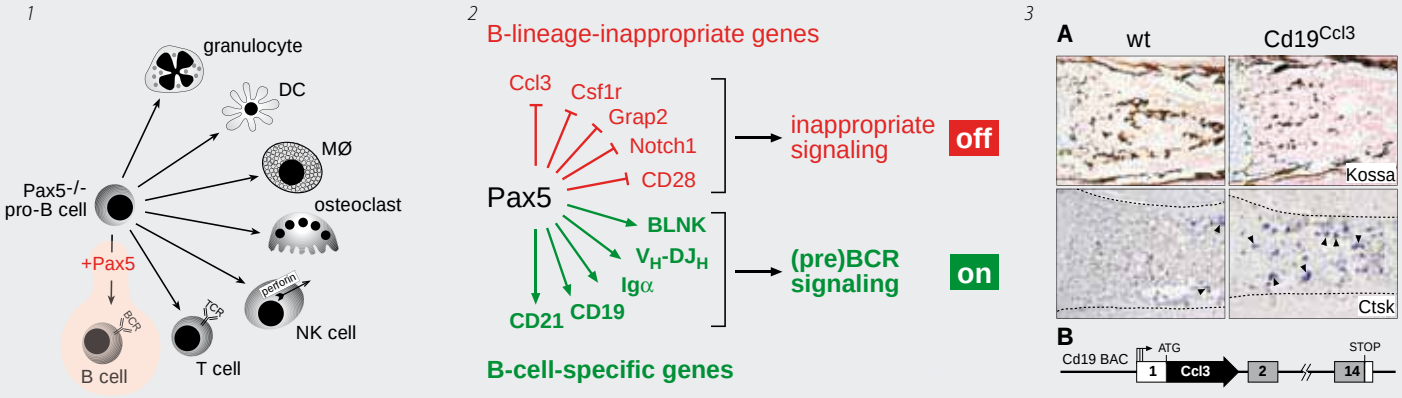

Figure 1: *B-lineage commitment by Pax5. Pax5$^{-/-}$ pro-B cells are early progenitor cells, which can differentiate along the indicated hematopoietic lineages with the exception of the B cell pathway.*

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

Figure 3: *Ectopic Ccl3 expression in B cells causes bone loss. (A) Bone phenotype of Cd19^{Ccl3} transgenic embryos. Adjacent sections through the hindlimb of transgenic and wild-type E18.5 embryos were analyzed by van Kossa staining (visualizing mineralized bone in black) and by in situ hybridization detecting expression of the osteoclast-specific cathepsin K (Ctsk) gene. Dashed lines indicate the contours of the bone. (B) Cd19^{Ccl3} transgene. The mouse Ccl3 cDNA was inserted into the B-cell-specific Cd19 gene.*

Spatial Control of VDJ Recombination

The immunoglobulin heavy-chain (*IgH*) locus contains ~200 V_H genes, which are spread over a 2.4-Mb region. The question therefore arises how the distal V_H genes are able to pair with the proximal D_H segments over such a large distance to undergo V_H-DJ_H recombination during pro-B cell development. Recently we demonstrated that Pax5 promotes the juxtaposition of distal V_H genes next to the proximal *IgH* domain. We refer to this phenomenon as locus contraction. In Pax5-deficient pro-B cells, the distal V_H genes are separated from the proximal *IgH* region by a large distance in the nucleus, whereas all *IgH* gene segments are colocalized in Pax5-expressing wild-type pro-B cells. Looping of individual subdomains is responsible for the contraction of the *IgH* locus, which facilitates V_H-DJ_H recombination of distal V_H genes. We now investigate the molecular mechanism by which Pax5 controls the contraction of the *IgH* locus.

T Cell Specification by Notch1

As signaling through the Notch1 receptor is essential for the initiation of T cell development in the thymus. Stromal cells ectopically expressing the Notch ligand Delta-like-1 mimic the thymic environment by inducing uncommitted Pax5-deficient progenitor cells to undergo efficient *in vitro* differentiation to immature CD4$^+$CD8$^+$ T cells. Notch-stimulated *Pax5*$^{-/-}$ progenitors rapidly down-regulate the expression of B-cell-specific genes, consistent with a role of Notch1 in preventing B-lymphopoiesis in the thymus. At the same time, Notch signaling induces expression of the transcription factor genes *GATA3* and *Tcf1*, indicating that Notch1 acts upstream of *GATA3* and *Tcf1* in early T cell development. We are currently performing cDNA microarray experiments to better define the role of Notch1 in T cell specification.

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TIM CLAUSEN

Molecular Mechanisms of Protein Quality Control and Stress Response

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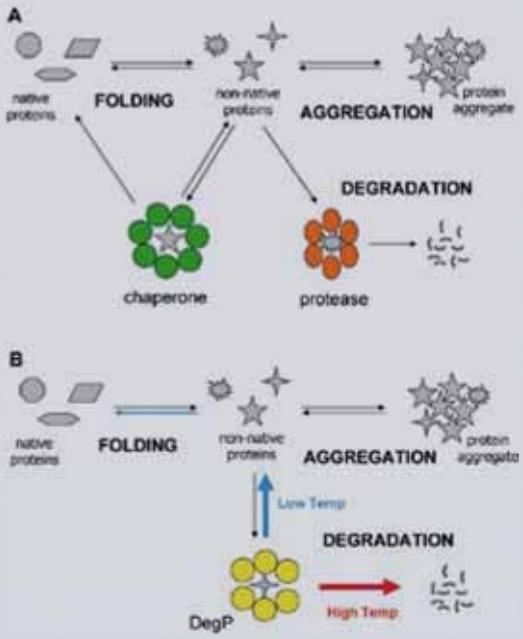

The misfolding and aggregation of protein molecules is a major threat to all living organisms. Cells have therefore evolved a sophisticated network of molecular chaperones and proteases to prevent protein aggregation (Figure 1A), a process that is regulated by multiple stress response pathways. We perform a structure-function analysis of several of these factors in order to better understand how cells deal with folding stress.

"Refoldable" or "Not Refoldable" - Is that the Question?

A combination of chaperone and protease function in a single protein could provide a direct and rapid response to protein folding problems. The heat shock protein DegP (HtrA) can switch between these dual functionalities in a temperature-dependent manner (Figure 1B) and thus offers unique possibilities for investigating how cells distinguish between proteins that can be refolded and "hopeless" cases that need to be degraded. DegP consists of a protease and two PDZ domains, which are prominent protein-protein interaction motifs. It is a widely conserved protein found in most organisms. Prokaryotic DegP has been attributed to the tolerance against various folding stresses as well as to pathogenity. Human homologs are believed to be involved in arthritis, cell growth, unfolded protein response, and apoptosis.

As we have shown by protein crystallography, the functional DegP hexamer is formed by the staggered association of two trimeric rings and represents a novel type of cage-forming protein (Figure 2). The six protease domains construct the top and bottom of the molecular cage, whereas the twelve PDZ domains generate the mobile sidewalls. We crystallized the "low temperature" chaperone form of DegP in both open and closed conformations. The transition between these two states is mediated by the inherent *en-bloc* mobility of the PDZ domains functioning as gatekeepers of the DegP cage. Thus, the PDZ domains might permit a direct coupling of substrate binding and subsequent translocation into the inner chamber. This two-step binding process is similar to that of other cage-forming proteins, such as the proteasome or Clp proteins.
The proteolytic sites of DegP are located in the inner cavity of the cage and can only be accessed by unfolded proteins. In the chaperone form, they were present in an inactive state, in which catalysis as well as substrate-binding were abolished. Thus it still remains elusive how DegP degrades unfolded proteins and how the switch in activity is accomplished. We will screen different DegP mutants for potent protease inhibitors that could stabilize the "high temperature" protease form and allow its crystallization. Furthermore, we plan to extend the DegP approach to eukaryotic homologs (e.g. plant DegP1) and to functionally related proteases containing PDZ domains like DegQ and Tsp.

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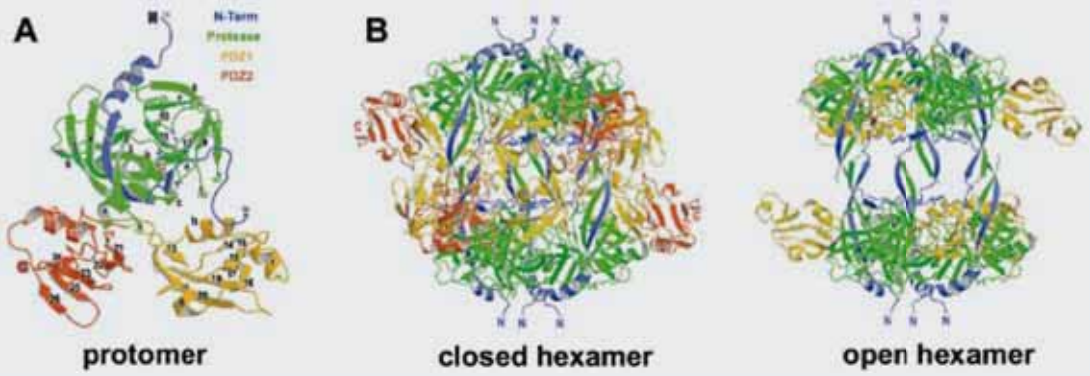

Figure 1: *Protein quality control. (A). The diagram illustrates the different fates of misfolded non-native proteins. According to the "kinetic partitioning model" of Gottesman and coworker, aggregate formation is counteracted by the action of proteases and chaperones. (B). DegP combines the antagonistic activities of a protease and a chaperone and thus guarantees a rapid cellular response to protein folding stress. The switch in activity is regulated by temperature. At low temperatures (<25°C) the protein acts as a chaperone, whereas at elevated temperatures the protease function is dominant.*

Figure 2: *Structure of DegP. (A). Ribbon presentation of the protomer, in which the individual domains are colored differently. Residues of the catalytic triad are shown in a ball-and-stick model.*
The nomenclature of secondary structure elements and the termini of the protein and flexible regions are indicated. (B). Side views of the DegP hexamer that was observed in two different forms - in a closed form and in a remarkable open form. The transition between both states is accomplished by the mobile PDZ domains.

Figure 3: *Structure of DegS. (A). Ribbon presentation of the DegS trimer (top view) with each subunit colored differently. (B). Diagram of DegS activation. In DegS, the PDZ domain obtains a regulatory function, offering a binding site for an allosteric activator.*

Stress Response by Regulated Intramembrane Proteolysis

In the extracytoplasmic stress response, the stress signal must cross a membrane and thus sophisticated pathways are required to transduce the signal from one compartment to another. A well-studied system is the bacterial σE stress response that is triggered by excessive amounts of unfolded proteins in the periplasm, particularly unfolded outer membrane porins. The alternative σ-factor σE is a transcriptional activator that directs the expression of several stress genes. Under non-stress conditions, the activity of σE is inhibited by RseA, a membrane spanning protein, whose cytoplasmic domain captures σE, thereby preventing σE from binding to RNA polymerase. Activation of σE is controlled by a proteolytic cascade that is initiated by the DegS protease. Upon folding stress, peptides corresponding to the C-terminus of misfolded outer membrane proteins serve as stress signals, bind to the PDZ domain of DegS and activate its protease function. Activated DegS cleaves RseA, thereby triggering the σE-response.

Recently, we determined the crystal structures of three distinct states of DegS from *E. coli*. DegS alone exists in a catalytically inactive form. Binding of stress-signaling peptides to its PDZ domain induces a series of conformational changes that activates protease function. Backsoaking of crystals containing the DegS-activator complex revealed the presence of a trypsin-like activation domain and demonstrated the reversibility of activation. Taken together, the structural data illustrate in molecular detail how DegS acts as a periplasmic stress sensor (Figure 3). The central regulatory element is the PDZ domain that offers a binding site for a peptidic cofactor and thereby couples the recognition of misfolded protein with the activation of the σE response.

Future structure-function studies are aimed at validating this model. Furthermore, we will start structural work on the second protease of the σE pathway, the membrane protease YaeL.

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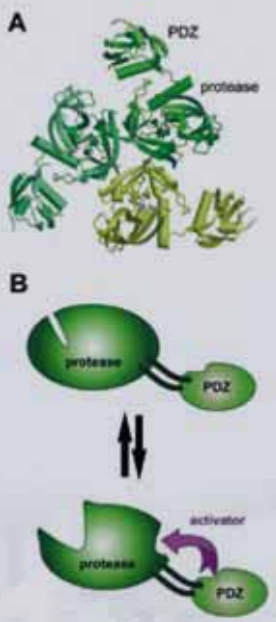

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CARRIE COWAN
Cell Polarity Establishment during Development

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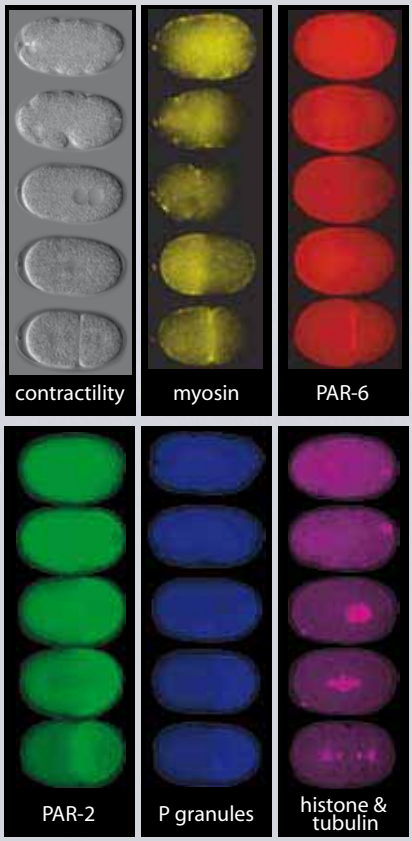

Carrie Cowan / Group Leader *(since December)*

Cell polarity allows for morphological and functional differentiation and is essential for all organisms. The general process of cell polarization is conserved among very different sorts of polarized cells. We use a combination of quantitative time-lapse microscopy, genetics, and micromanipulations in C. elegans embryos to investigate the molecular mechanisms controlling the establishment of cell polarity during development.

Cell Polarity in *C. elegans* Embryos

Cell polarity is the spatial asymmetry of cellular content and function. Despite the range of processes in which cell polarity is important, the general mechanism of polarity establishment is the same for most cells. First, a signal provides spatial and temporal information for polarization. Second, the signal is propagated to form distinct domains within the cell which define the polarity axis. Third, the domains communicate downstream to the cellular contents to allow for functional differences. *C. elegans* embryos begin with no polarity. Polarity is established in one-cell embryos by the formation of the anterior-posterior body axis of the worm (Figure 1). The anterior-posterior axis is defined by the segregation of PAR proteins into two distinct cortical domains, each domain occupying half the embryo. The cortical PAR domains control the unequal segregation of cell fate determinants during cell division, therefore the daughter cells differ in developmental fate. Cells arising from the posterior will become the germ cells of the adult worm, while cells arising from the anterior will form the rest of the adult tissues.
C. elegans embryos are an excellent system for investigating cell polarity. We are studying the molecular mechanism of polarity establishment using RNAi-based mutant screens, quantitative time-lapse microscopy and mechanical manipulations, such as laser ablation and optical trapping.

What is the Polarity Establishment Signal?

Using a combination of genetic mutants and laser ablation, we have demonstrated that the establishment of polarity depends on the centrosome. The most prominent role of centrosomes in most cell types is microtubule nucleation, but the centrosome does not need microtubules to induce polarity in *C. elegans* embryos. The requirement for the centrosome is transitory: once polarity has been initiated, the centrosome is dispensable for polarization. Centrosomes appear to provide a signal to break the symmetry of one-cell embryos. We would like to identify the molecular mechanism of the centrosomal signal.

What Tells the Embryo when and where to Polarize?

Timing of centrosome assembly correlates with the timing of polarity establishment, and defects in timing of centrosome assembly lead to a failure in polarity establishment. The centrosome lies very close to the cortex at the time of

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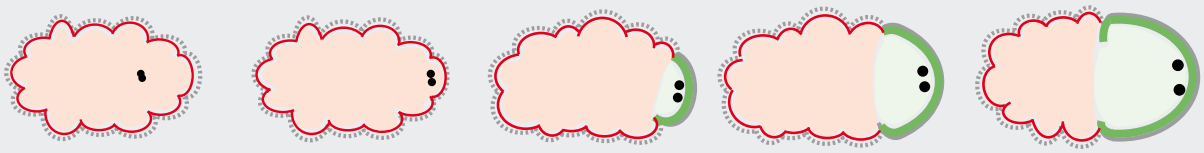

Figure 1: *Polarity establishment in one-cell embryos. About 30 minutes after fertilization, the symmetry of a one-cell embryo is broken when the anterior-posterior axis is established. Polarity establishment involves an asymmetric distribution of the acto-myosin network (yellow) and contractility (gray), which facilitate the segregation of PAR proteins (red and green) into two distinct domains. The PAR proteins determine the segregation of fate determinants (blue) and posterior displacement of the mitotic spindle (pink). The images are from the time of polarity establishment to cytokinesis, a period of only 20 minutes.*

Figure 2: *Centrosomes provide a signal to establish polarity. Centrosomes (black dots) are adjacent to the cell cortex at the time of polarity establishment. The posterior domain (green) expands from the centrosome position until the posterior domain occupies half the embryo; the anterior domain (red) shrinks at the same time.*

Figure 3: *Segregation of polarity during cell division. The anterior-posterior polarity of one-cell embryos is reiterated over several cell divisions during early embryogenesis, similar to stem cell propagation. The exclusive segregation of polarity requires that PAR polarity is precisely matched to the position of cell division. Coordination of domain size with cleavage requires significant changes in the size of the anterior and posterior domains, best observed by the diminishing size of the posterior (green) domain during development.*

polarization, and the posterior domain expands uniformly from the centrosome position (Figure 2). We are investigating how the centrosome position influences the spatial regulation of polarity establishment using mechanical manipulations and mutant analysis.

How does the Polarity Signal make a Cortical Domain?

The establishment of anterior and posterior PAR protein domains relies on differences in the contractility of the acto-myosin cortex: the anterior cortex ruffles but the posterior cortex is smooth. The establishment of anterior-posterior contractile polarity relies on the centrosome. Thus, the centrosome signal must integrate into pathways that regulate the acto-myosin cortex. What is the molecular connection between the centrosome and the cortex? We have used RNAi-based phenotypic screens to identify genes that affect the establishment of acto-myosin contractile polarity. We would like to determine how these molecules interact with the centrosome signal using genetics and biochemistry.

How is Cell Polarity Segregated during Development?

In *C. elegans* embryos during cell division, the anterior and posterior PAR domains are inherited exclusively by the anterior daughter cell and the posterior PAR proteins are inherited exclusively by the posterior daughter cell (Figure 3). This exclusive segregation of polarity requires that the position of the PAR domain boundary is coordinated with the position of the cell cleavage. We have uncovered a PAR domain correction mechanism acting at anaphase that ensures the PAR domain boundary is precisely matched to the cytokinesis furrow. The anterior and posterior domains shrink or expand to match mispositioned cytokinesis furrows. The correction mechanism depends on molecules required for positioning the furrow in response to spatial information provided by the spindle poles/centrosomes, suggesting that centrosomal signals are required for PAR domain correction. We are using genetics and mechanical manipulations to understand how spindle poles/centrosomes rearrange the PAR domains during cell division.

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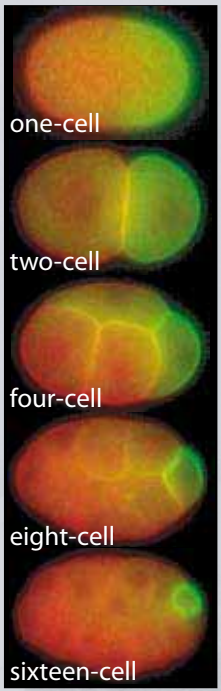

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BARRY DICKSON
Genes, Circuits and Behaviour

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We are using molecular genetic techniques to investigate how genes regulate the assembly, function, and plasticity of defined neural circuits, and how the activity of these circuits governs specific behaviours. Our research is focused on the fruit fly, Drosophila melanogaster, because it offers both powerful methods for the genetic analysis of neural circuits and a sophisticated behavioural repertoire.

Genes

Seymour Benzer pioneered the hunt for genes that influence behaviour in his ground-breaking work with *Drosophila* during the 60s and 70s [1]. One fascinating behavioural gene, studied by Benzer, Jeff Hall, Bruce Baker, Daisuke Yamamoto, and others, is the *fruitless (fru)* gene. These researchers established that *fru* plays a critical role in male courtship behaviour, and showed that the *fru* gene is alternatively spliced to produce distinct male- and female-specific transcripts (Figure 1). We have recently shown that male-specific splicing of *fru* is not only necessary but also sufficient for male courtship behaviour [2], aggression, and sexual orientation. That is, females engineered to splice *fru* in the male mode both court and fight like males, with courtship directed towards other females (Figure 2) and aggression directed towards males. How do the male *fru* products direct this switch in sexual behaviour? They are thought to encode a set of transcription factors, and so presumably that act by regulating the expression of other genes. We are now trying to find out when and where the *fru* proteins act, which genes they regulate, and what these target genes do.

Classical forward genetic approaches to behaviour are hampered by the fact that any given gene is likely to have a variety of functions in different tissues. This makes it difficult to dissect out any specific role it might have in behaviour. For example, a homozygous mutant might misbehave only as an indirect consequence of unrelated defects (e.g. metabolic defects), or unrelated phenotypes might obscure an interesting behavioural phenotype (e.g. developmental lethality). To try to overcome this problem, we have developed a genome-wide transgenic RNAi library in *Drosophila*, allowing us – at least in principle – to disrupt the function of any gene specifically in the cells or tissues of interest. Thus, we can now systematically search for genes required in specific neurons for specific behaviours, regardless of any additional functions these genes might have in other tissues. We are currently screening for genes required in the nervous system for male and female mating behaviour, and together with Krystyna Keleman we are also looking for genes involved in learning and memory.

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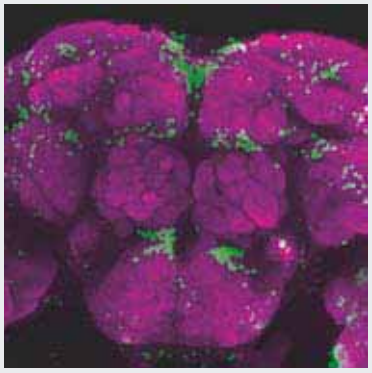

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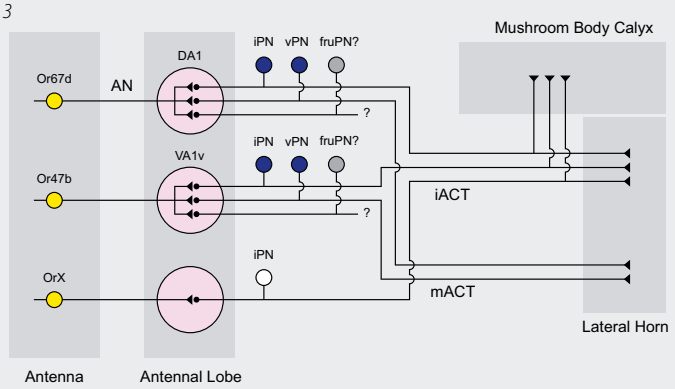

Figure 1: *fruitless expression in the male brain. An image of a male brain, stained for male-specific fru products (Fru^M, green) and synapses (magenta).*

Figure 2: *Switching sexual behavior. A female engineered to express Fru^M (bottom) courts a wild-type female (top).*

Figure 3: *Olfactory circuits for pheromone detection. Schematic of olfactory pathways. Yellow indicates ORNs in the antenna. OrX is a generic ORN. Pink indicates glomeruli in the antennal lobe, and blue the projection neurons (PNs) that convey olfactory signals from the antennal lobe to the lateral horn and mushroom body calyx. Grey indicates a putative class of fru-expressing PNs. AN, antennal nerve; iACT, inner antennocerebral tract; mACT, middle antennocerebral tract.*

Circuits

Genes impact behaviour by directing the assembly, function, or plasticity of neural circuits. To understand the links between genes and behaviour, we first need to define the relevant neural circuits and their genetic constitution. One major focus in the lab is to map out the circuits involved in male courtship and aggression, using the expression of the *fru* gene as a genetic marker for the relevant neurons [3]. In particular, we are concentrating on the olfactory pathways that mediate responses to sex pheromones during mating behaviour, and possibly also aggression (Figure 3). We have found that olfactory receptor neurons (ORNs) expressing the odorant receptor Or67d mediate physiological and behavioural responses to the male sex pheromone *cis*-vaccenyl acetate. Another set of neurons expressing the receptor Or47b may detect a different, and still unidentified, pheromone. We are currently examining the anatomy and function of the higher-order olfactory pathways activated by these two classes of ORN (Figure 3).

Behaviour

Complex behaviours, such as courtship and aggression, can be broken down into discrete sub-behaviours. The behaviour unfolds as the fly progresses through these distinct component states in response to various environmental stimuli. To help analyse such behaviours, we are collaborating with Wilfried Grossmann (University of Vienna) in an effort to develop computational methods for the automatic recognition of distinct behavioural states and the transitions between them. With such tools, we should be able to study behaviour objectively, quantitatively, and at high spatial and temporal resolution. Such methods are indispensable if we are to understand how individual genes and neurons act in concert to sculpt a complex behaviour.

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FRANK EISENHABER

Discovering Biomolecular Mechanisms with Computational Biology and Protein Biochemistry

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In-depth theoretical analysis of biomolecular sequence information and of other high-throughput experimental data is a productive approach to gain mechanistical insight into biological processes at the molecular and the cellular level. Especially protein sequence analytic methods can reveal considerable insight. If the functional predictions are combined with targeted verification experiments either in collaborating groups or within our biochemical lab, breakthroughs become possible.

Our mission involves both collaborative research activities with experimental groups at the IMP and interacting institutions as well as the realization of genuine bioinformatics research projects in the field of genome text interpretation. Since the foundation of the group in 1999, the group's success stories include (i) discoveries of new biological mechanisms with theoretical methods, (ii) the development of algorithms and software for protein sequence analysis and protein mass spectrometry and (iii) the refinement of experimental assays for lipid posttranslational modifications and the biochemical verification of a variety of predicted protein functions.

Discovering new Biological Mechanisms with Computational Biology

During its history at the IMP, the group has been instrumental in a number of ground breaking discoveries, a few of which are listed here. The prediction of SET domain methyltransferase activity paved the way for the discovery of the suv39h-dependent histone methylation and the start of biochemical epigenetics. The discovery of ATGL, the enzyme at the surface of lipid drops for hydrolysis of triglycerides, closed a gap in the mammalian fat catabolism pathway. The discovery of the kleisin protein family linked chromatin organization processes in chromosome segregation, condensation and DNA repair mechanistically. Our sequence-analytic work gave the critical hints for the discovery of the docking mechanism for substrate-specific subunits of the APC complex. These and dozens of other cases of successful collaborations reside on two cornerstones: (a) on qualified and tightly interacting specialists for sequence and biological data analysis that operate upon request besides their own topic-oriented work and (b) on time-efficient protein sequence analyses based on the "ANNOTATOR" suite.

Development of new Algorithms and Software Packages for Protein Sequence Analysis and Prediction of Function

Our group is known for its highly accurate predictors for lipid posttranslational modifications (GPI lipid anchoring, myristoylation, farnesylation, geranylgeranylation), for PKA phosphorylation sites, protein convertase cleavage sites and for peroxisomal localization translocation signals. Large-scale database studies together with individual experimental efforts support the notion of hidden

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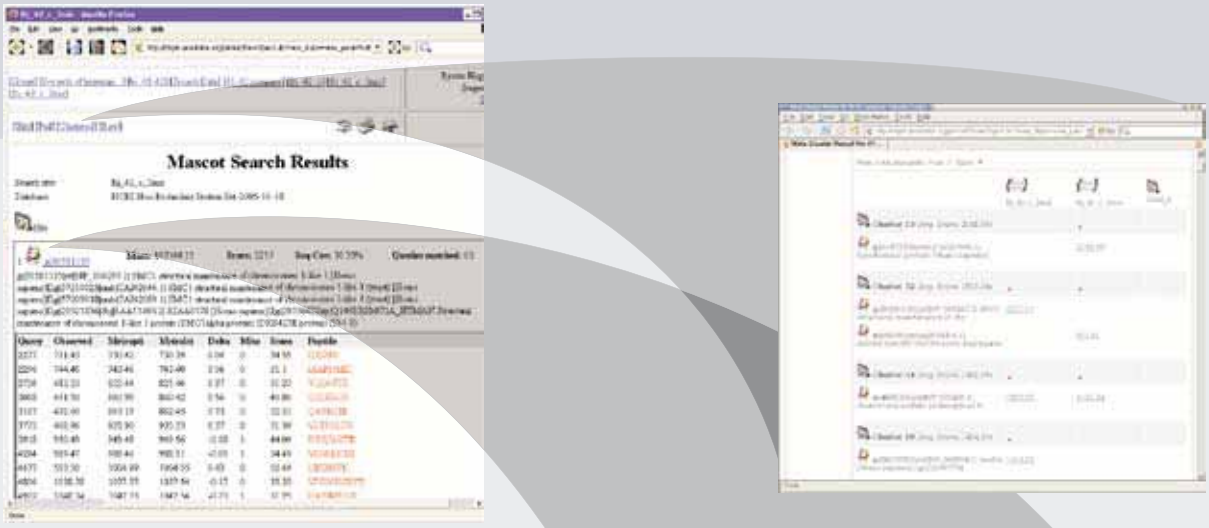

Figure 1: *MS-ANNOTATOR: From protein hit lists to complex composition and to protein function analysis. The MS-ANNOTATOR is a software environment for the efficient analysis of mass spectrometry results for protein identification. The pipeline uploads raw data from the instruments, performs the necessary format transformations, and executes the MS Cleaner for noise removal and MASCOT for the interpretation of the remaining spectra. The final results are displayed as a protein list for each measurement (left insert) and as protein-complex information (right insert; essentially identical sequences are clustered and protein contaminants are suppressed). The entire sequence-analytic assortment of methods can by accessed with a mouse click and the analysis results are displayed together with the peptides (in yellow) that were found in the MS/MS experiment (insert in the middle).*

signals for translocation and posttranslational modifications; i.e., targets with *in vivo* functional sequence signals that remain unused due to the inappropriate biological context in normal physiology.

The ANNOTATOR/NAVIGATOR suite has reached maturity and has become the major tool for protein sequence analysis within the group and collaborators. More than 30 sequence-analytic tools as well as composed algorithms are bundled in the ANNOTATOR so that they are automatically executed over queries if suitable, the results are processed and presented in a condensed manner to the researcher. Even the automated collection of homologous protein families containing tens of thousands of members and their analysis is possible within the ANNOTATOR framework. The NAVIGATOR function allows retrieving information relevant for the project from diverse biological databases. A number of these services are available also through the Internet node (http://mendel.imp.univie.ac.at).

Software for Protein Mass Spectrometry

A typical protein identification experiment with the MS/MS methodology leads to ca. 5000 spectra each with ca. 300 peaks. For the final interpretation, just 1% of the spectra are of significance and only about 30 peaks per spectrum support the interpretation. The program MS Cleaner is a valuable shortcut for noise removal; it recognizes 50-65% of the spectra as non-relevant for peptide recognition and reduces the number of peaks in the remaining spectra by about one-fourth. The MS-ANNOTATOR has now integrated the workflow from raw protein tandem mass spectrometry data over background removal and data interpretation to sequence annotation of protein hits (see Figure 1).

Request-based Biochemical Studies for Verifying Sequence-based Predictions

Sequence-analytic predictions for proteins typically involve molecular-functional properties such as enzymatic activities, ligand-binding properties or posttranslational modifications. Especially small collaborating groups have difficulties in following up sequence-based predictions that are relevant for their research. We gradually accumulate a set of specialized biochemical methods for this purpose. The recently developed Benetka-Koranda method for testing protein prenylation was further advanced for a few other lipid modifications and applied on protein targets with predicted capability for lipid posttranslational modifications.

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CHRISTINE HARTMANN
Formation and Patterning of the Vertebrate Skeleton

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The skeleton is essential for the vertebrate organism; it supports the body, provides the mechanical framework for physical movements, and protects internal organs. During embryonic development, the sides where the future skeletal elements (bones) are formed, their size and the formation of the articulations (joints) has to be controlled. At the beginning, most of the skeletal elements are formed as cartilaginous templates and therefore need to be remodelt into bony tissue. We use mouse and chick as model organisms to gain insight into how these different processes are regulated during both embryonic and postnatal development. In particular, we investigate the role of Wnt-signaling in skeletogenesis.

Regulation of Chondrocyte Maturation and Bone Homeostasis

In vertebrates, the Wnt-gene family comprises 19 members, all of which encode secreted signaling molecules. Wnts utilize different intracellular signaling pathways. The best studied one is the canonical Wnt-signaling pathway with β-catenin being the key component. At least nine Wnt genes are expressed during skeletal development in chondrocytes, osteoblasts, and in the joint region. Many of the Wnt-genes have been knocked-out in mouse, but only the loss-of Wnt5a causes skeletal defects. Since some of the Wnts overlap in their expression patterns, it is likely they might act redundantly, explaining the observed lack of a phenotype in some single knock-outs. In order to address the role of canonical Wnt-signaling in skeletogenesis, we used a conditional gene targeting approach to either delete or stabilize the key-component, β-catenin. In addition, we analyzed the loss-of function phenotypes of the Wnt-ligands, Wnt9a and Wnt4, which are both expressed in joints. The resulting knock-out and gain-of function phenotypes revealed a role for canonical Wnt-pathway in chondrocyte and osteoblast differentiation, and the formation and maintenance of joints (Figure 1). We have shown that too much β-catenin results in a loss of chondrogenic and osteogenic lineages. While loss-of β-catenin does not affect the differentiation of mesenchymal cells into chondrocytes, no mature osteoblasts differentiate from mesenchymal cells lacking β-catenin. Their precursors, osteochondroprogenitor cells, differentiate into chondrocytes instead (Hill et al., 2005). The three cell types, chondrocytes, osteoblasts and joint cells share the same mesodermal origin (Figure 2), suggesting that the observed alterations upon loss-of β-catenin activity could be caused by a common mechanism. Using ex vivo cell culture systems, we are currently investigating the underlying molecular changes.

The various skeletal elements that make up the vertebrate skeleton differ in size and shape, but little is known about the molecular mechanisms controlling these two features. Interestingly, alterations in the process of chondrocyte maturation often lead to changes in the size of skeletal elements. Studying the Wnt9a knock-out animals, we uncovered a very specific requirement for

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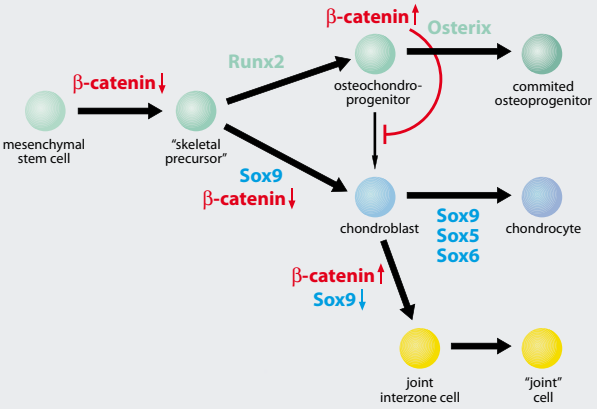

Figure 1: *Skeletal preparations of a wild-type (left) and conditional β-catenin knock-out mouse (right). Cartilaginous regions are stained blue; mineralized areas are stained red.*

Figure 2: *β-catenin levels need to be modulated during skeletal lineage differentiation: only mesenchymal cells expressing low levels of β-catenin can differentiate into skeletal precursor which will give rise to osteoblasts, chondrocytes, and cells of the joint. Within the osteoblast and joint lineage β-catenin levels need to be raised to enable differentiation into osteoblasts and cells of the joint, respectively. While within the chondrocytic lineage, β-catenin levels need to be maintained at a low level. Furthermore, after joint formation, continuous Wnt-signaling by Wnt9a and Wnt4 is required to maintain the identity of joint cells.*

Figure 3: *Wnt9a expression in mature synovial joints of the hand visualized by β-galactosidase staining (green).*

Wnt9a during long-bone development: Wnt9a controls the expression of a central regulator of chondrocyte maturation in a spatio-temporal manner. Thus sequential activation, or temporal recruitment of regulatory molecules, which themselves, like Wnt9a, control a central regulator of chondrocyte maturation, are likely to be involved in fine-tuning the skeletal element size. Preliminary results suggest that Wnt9a employs two different intracellular signaling pathways. We are currently investigating this possibility further, using genetic, biochemical and cellular approaches.

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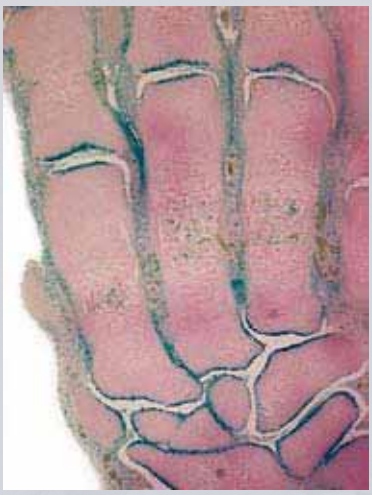

Synovial Joint Development

In gain-of-function analysis in chicken, Wnt9a was identified as a major player in the induction of synovial joint development (Hartmann and Tabin, 2001). In mouse, Wnt9a is expressed in the early joint interzone and, after the formation of the synovial joint, in the articular cartilage and in the cells of the joint capsule (Figure 3). In mice lacking Wnt9a, an ectopic cartilage nodule differentiates from synovial cells in the humeral-radial joint. In humans, this is known as synovial chondroid metaplasia. However, loss-of Wnt9a activity did not lead to any defects in early joint formation. Thus the phenotype points to a role for Wnt9a in the maintenance of joint integrity. Two other Wnt-genes are expressed in the joint regions: Wnt4 and Wnt16. Wnt4; Wnt9a double-mutant mice, which show synovial chondroid metaplasia in two additional joints and fusions of carpal and tarsal elements (Später, et al., 2006).

Our long-term goal is to identify regulators of Wnt9a as well as target genes downstream of the Wnt9a signaling pathway. Using a transgenic approach in combination with the search for evolutionarily highly conserved genomic regions within the Wnt9a locus, we aim to identify regulatory elements responsible for Wnt9a expression in the early joint interzone. Identification of such a joint-specific element would provide a useful tool to screen for factors that are necessary for initiation and regulation of Wnt9a expression in the joint-forming region and will inevitably allow us to better understand how the skeleton is patterned.

THOMAS JENUWEIN

Epigenetic Control by Histone Methylation

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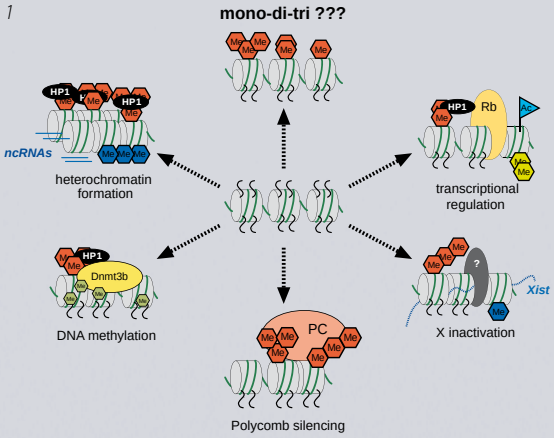

Epigenetic mechanisms, such as histone modifications, control eukaryotic development beyond DNA-stored information. The characterization of histone methyltransferases (HMTases) strongly established histone lysine methylation as a central epigenetic modification for the organization of eukaryotic chromatin with far-reaching implications for proliferation, cell-type differentiation, stem-cell plasticity, gene expression, genome stability and cancer.

The Indexing Potential of Histone Lysine Methylation

Histone lysine methylation has been linked with constitutive heterochromatin formation, X inactivation, Polycomb-group (PcG) dependent repression and epigenetic gene regulation at euchromatic positions (Figure 1). Each methylatable lysine residue in the histone N-termini can exist in a mono-, di- or tri-methylated state, thereby extending the coding potential of this particular histone modification. Using highly specific methyl-lysine histone antibodies together with quantitative mass-spectrometry, we could show that pericentric heterochromatin is selectively enriched for H3K9me3 and H4K20me3. This profile is dependent on the 'heterochromatic' Suv39h HMTases and on novel Suv4-20h enzymes. The Suv4-20h HMTases are nucleosome-specific and require a pre-existing H3K9me3 mark for their recruitment to heterochromatin, thus revealing a silencing pathway for the induction of combinatorial histone lysine methylation imprints. By contrast, facultative heterochromatin at the inactive X chromosome (Xi) is characterized by a different methylation pattern (H3K27me3 and H4K20me1) (Figure 2) that is mediated by the PcG enzyme Ezh2 and another, currently unknown, HMTase. Our data underscore the combinatorial coding potential of histone lysine methylation as epigenetic landmarks in eukaryotic chromatin.

Epigenetic Reprogramming by Histone Lysine Methylation

Currently, more than 50 distinct modifications (acetylation, methylation, phosphorylation, etc.) have been described in the N-termini of the four core histones. Intriguingly, there is an under-representation of repressive marks in quiescent (resting) cells and in stem and regenerating cells, but a selective accumulation of aberrant histone lysine methylation profiles in aging, 'stressed' and tumor cells (Figure 3). These data suggest that distinct histone lysine methylation profiles contribute to the epigenetic 'make-up' of stem cells vs. more committed cells. To test this hypothesis, we have generated mutant mice that lack crucial HMTases, such as the Suv4-20h enzymes. In addition, we have screened chemical libraries (in collaboration with Boehringer Ingelheim, Ridgefield, USA) and identified a small molecule inhibitor for the G9a HMTase. Finally, we have been characterizing jumonjiC-containing proteins that represent demethylases with the potential

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[7] *until May*

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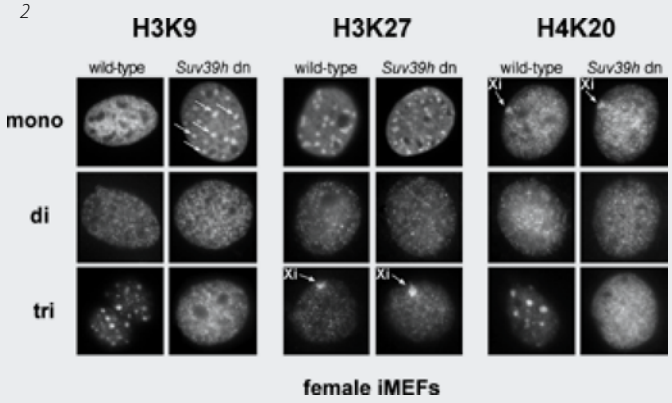

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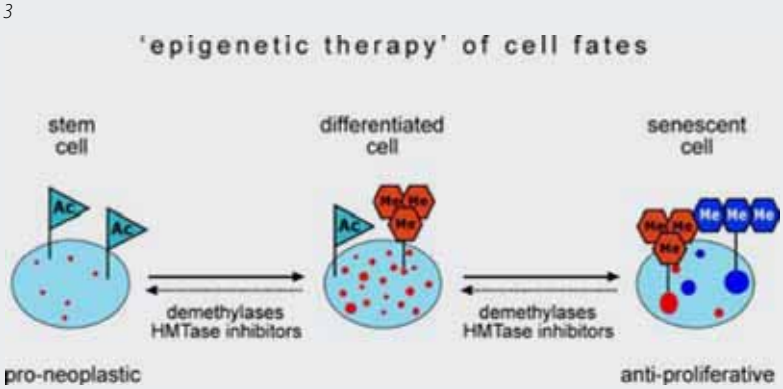

Figure 1: *The many faces of histone lysine methylation. The figure summarizes described roles of histone lysine methylation in major epigenetic paradigms. Distinct lysine positions are shown as colour-coded hexagons to indicate H3K4 (green), H3K9 (red triangular), H3K27 (red diagonal) and H4K20 (dark blue). DNA methylation is depicted by small orange hexagons.*

Figure 2: *Distinct methylation states are epigenetic landmarks in mouse interphase chromatin. Female wild-type and Suv39h dn iMEFs were stained with methyl-lysine histone antibodies that discriminate mono-, di- and tri-methylation of H3K9, H3K27 and H4K20. Foci of pericentric heterochromatin that were visualized with DAPI (not shown) are decorated by H3K9me3, H3K27me1 and H4K20me3 in wild-type nuclei. By contrast, the inactive X chromosome (Xi) is enriched for H3K27me3 and H4K20me1. The occurrence of H3K9me1 at pericentric heterochromatin in Suv39h dn nuclei is indicated by multiple arrows.*

Figure 3: *An 'epigenetic therapy' for cell fate specification. Normal, differentiated cells are characterized by a balanced representation of active (e. g. acetylation; blue flag) and repressive (e. g. lysine methylation; red and blue hexagons) histone modifications. In senescent or aged cells, repressive modifications accumulate at large blocks of 'ectopic' heterochromatin. By contrast, stem cells display a general under-representation of repressive histone modifications. These global histone modification patterns can be used as diagnostic markers for cell fate specification. In addition, they suggest a possible 'epigenetic therapy', in which demethylases and HMTase inhibitors may allow the reprogramming of senescent cells or, on the other hand, drive tumor cells into information overflow, chromatin catastrophe and apoptosis.*

to remove repressive H3K9me3 marks. Together, these approaches promise to yield new insights into the plasticity of cell fate decisions and may offer novel strategies for exploratory research to modulate tissue regeneration and to revert aberrant development.

An Epigenetic Map of the Mouse Genome

Alterations in the chromatin structure represent the key epigenetic principle to organize the information stored in the genome. In the context of the Austrian GEN-AU initiative (www.gen-au.at) and the European Network of Excellence (NoE) *'The Epigenome'* (www.epigenome-noe.net), we have initiated the large-scale and high-resolution analysis (CHIP-on-chip) of epigenetic transitions along entire mouse chromosomes, e.g. Chr 17. The data indicate that there are around 200 'heterochromatic islands' on mouse Chr 17 that are primarily localized in intergenic regions. We propose that these heterochromatic islands facilitate the partitioning of transcription units vs. intergenic regions and are important for the architecture and identity of mammalian chromosomes. We will continue this epigenetic landscaping by comparing chromatin from ES cells, differentiated cells and tumor cells. This project will lead to the establishment of reference epigenetic maps of the mouse genome, as it is organized under distinct developmental options.

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LUDGER KLEIN
T Cell Tolerance

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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 aim is to understand how selection processes during T cell development in the thymus contribute to the generation of a self-tolerant T cell repertoire through removal of potentially dangerous T cells, but as well through the induction of so-called suppressor T cells.

Suppressor T Cells

It is well established that the encounter of self-antigen during intrathymic development can lead to the "suicide" of potentially dangerous, autoreactive T cells. However, some T cells with specificity for self-antigens are spared from deletion and instead differentiate into so-called regulatory or suppressor T cells ($T_S$cells). The parameters that influence the choice between these mechanisms of tolerance are not understood. One of our goals is to elucidate the developmental cues (stromal interaction partner, signal strength, maturation state) that determine whether an autoreactive T cell is removed from the repertoire (negative selection) or differentiates into a suppressor T cell. In particular, we are trying to dissect the respective role of the cortical versus medullary compartments of the thymus using a combination of T cell receptor and antigen transgenic mice, whereby the fate of "self-specific" T cells in various experimental conditions can be followed.

"Promiscuous" Expression of Self-antigens in the Thymus

Deletion or re-programming of T cells upon encounter of self-antigens during intrathymic development is a cornerstone of immunological self-tolerance. However, it has remained questionable whether these mechanisms cover the entire spectrum of self-antigens, e.g., in how far such mechanisms could operate for tightly regulated tissue-specific proteins. We found that the range of self-antigens expressed in the thymus is surprisingly broad. This so-called "promiscuous" intrathymic expression of otherwise strictly tissue-specific proteins is confined to medullary epithelial cells (mTEC). The mechanistic basis for this phenomenon

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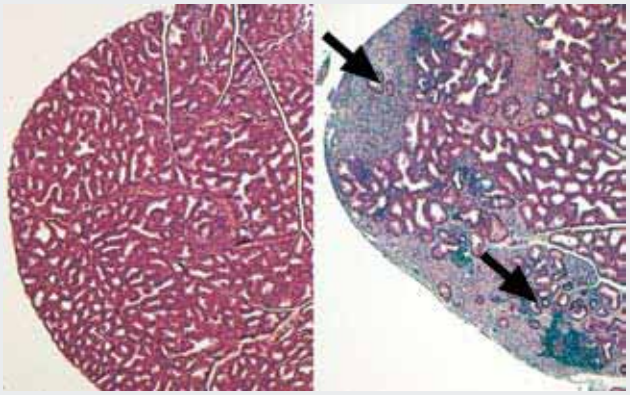

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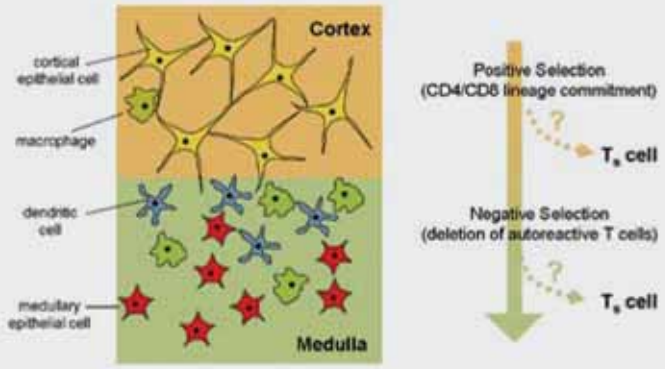

Figure 1: *Autoimmunity as a consequence of altered T cell selection in the thymus. The left panel shows the normal histological appearance of the harderian gland in wild-type animals. In the right panel, the harderian gland of an animal carrying a genetic lesion in thymic epithelium that affects T cell selection is shown. Autoimmune T lymphocytes have entered the organ, leading to tissue-destruction (arrows).*

Figure 2: *During their maturation in the thymus, developing T cells migrate from the outer cortex to the medulla in a temporally and spatially highly ordered manner. While positive selection for self-MHC restriction occurs upon interaction with cortical epithelial cells, negative selection (removal of autoreactive T cells) is thought to be largely confined to the medulla as a consequence of interactions with dendritic cells or medullary epithelial cells. The developmental stage at which the CD25+ lineage of suppressor T cells (Ts) branches off from "mainstream" T cell development remains to be elucidated. We are currently developing transgenic model systems to address this issue.*

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).*

(e.g. specific induction versus de-repression of particular genes) is only poorly understood. It was shown that the *Autoimmune Regulator (aire)* gene, a putative transcription factor that is specifically expressed in a not yet characterized subset of mTEC, is involved in "promiscuous" gene expression. Targeted disruption of *aire* leads to reduced expression of numerous self-antigens in mTEC, and *aire-/-* mice develop spontaneous autoimmunity. We have initiated a project which aims (i) to identify, isolate and characterize by flow-cytometry mTEC that express *aire*, and (ii) to study the consequences of antigen-expression in *aire*-expressing cells (deletion versus induction of anergy/suppressor function) by crossing these animals to T cell receptor transgenic animals

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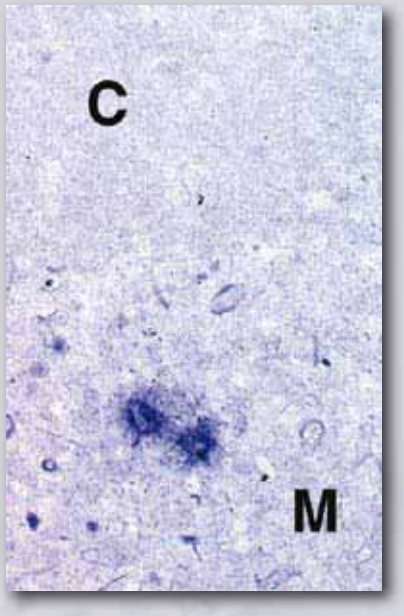

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THOMAS MARLOVITS
Design and Function of Molecular Machines

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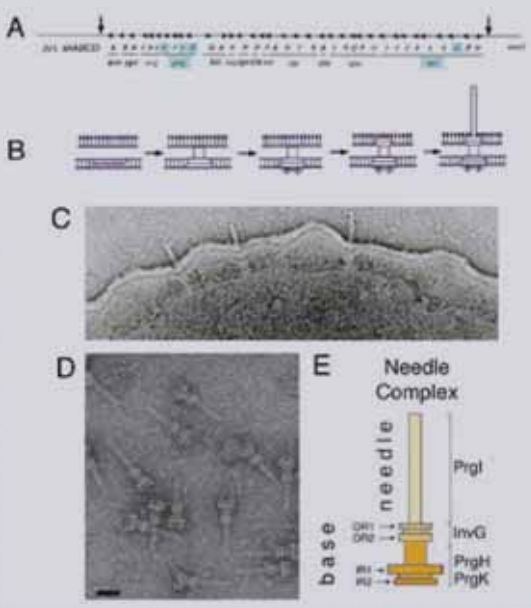

What keeps cells and organisms alive are specific functions performed by highly organized macromolecular assemblies. Our research is motivated to understand the fundamental design and function of such macromolecular complexes. In particular, we are interested in systems that are involved in protein transport and signaling. At the center of our research, our laboratory takes an integrated approach and combines tools for structural and molecular biology, biochemistry and biophysics. In particular, we use three-dimensional electron cryomicroscopy and image processing as a platform to link high-resolution structural biology with cell biology in order to obtain for the first time a mechanistic view of macromolecular machines from an atomic to cellular level.

Host-Pathogen-Interaction

Gram negative pathogens, like *Salmonella, Yersinia,* or *Shigella,* use the type III secretion system (TTSS) to initiate infection in eukaryotic cells. The TTSS is a complex macromolecular system that serves as a structural platform to make physical contact between cells and mediates the unidirectional transport of bacterial toxins (effector proteins) into eukaryotic cells. These systems are essential for a successful infection resulting in well-known clinical symptoms ranging from mild headaches and diarrhea to even life-threatening diseases, such as typhoid fever or bubonic plaque. Comprised of more than twenty proteins, TTSSs assemble into large "molecular nanomachines" composed of a set of soluble as well as membrane proteins. All of the structural components as well as other proteins involved in assembly and function are encoded on specific pathogenicity islands (Fig 1A). Recently, genetic and biochemical analysis revealed that assembly of the TTSS is a step-wise process during which stable intermediate substructures are formed (Figure 1B).

The Molecular Design

The most prominent substructure of the TTSS is known as the "needle complex", a cylindrical, needle-shaped and membrane-embedded organelle protruding from the bacterial envelope (Figure 1CD). The needle complex is believed to serve as a conduit for the safe transport of virulence factors from the bacterial cytoplasm through a number of natural barriers into eukaryotic cells. In *Salmonella typhimurium,* which serves as our model for bacterial delivery systems**,** this complex is formed by multiple copies of only five proteins: PrgH, PrgK, and InvG make up the membrane associated base-structure, PrgJ, the inner rod, and PrgI, the needle filament extending into the extracellular environment (Figure 1E and 2D). In order to investigate the molecular mechanism of type III secretion, we first set out to determine structural components of the TTSS. We were challenged by the mega-dalton size of the complex, its natural composition (membrane and soluble proteins), and the limited availability. Nevertheless, we were able to

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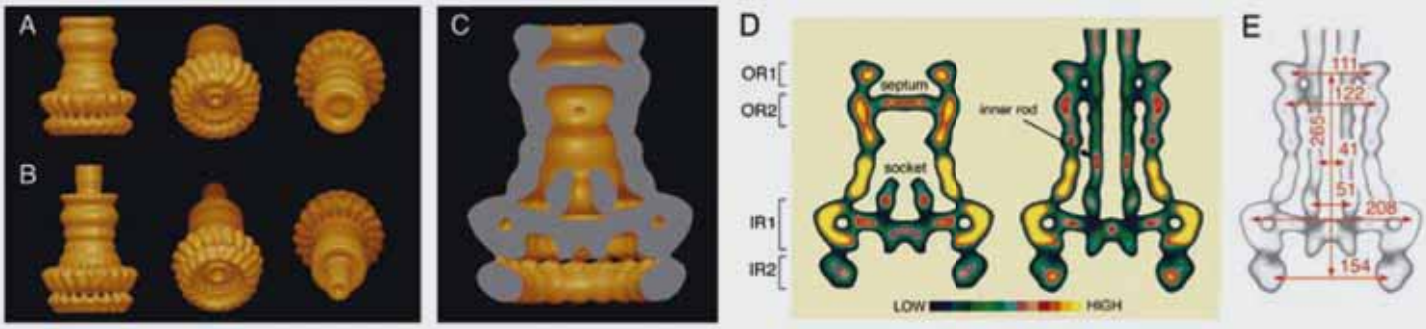

Figure 1: *The type III secretion system (A) The type III secretion system is located on the bacterial chromosome but encoded on a specific pathogenicity island. Structural proteins of the needle complex are indicated in blue color. (B) Formation of stable intermediate substructures during assembly. (C) Electron micrograph of osmotically shocked S. typhimurium showing the needle complex embedded in the bacterial envelope and released after detergent treatment (D) bar: 100 nm. (E) Schematic representation of the Salmonella needle complex and its components. PrgH, PrgK, and InvG make up the membrane embedded base structure, whereas PrgI forms the helical filament protruding into the extracellular environment. The inner rod (see Figure 2D) anchors the filament into the base.*

Figure 2: *The structure of the base and the needle complex Surface renderings of the base (A) and the needle complex (B) show that their overall shape is quite similar. However, tilted views indicate that during the assembly, individual domains must undergo large structural rearrangements. (C) The inside of the base reveals an internal structure (socket), which serves as a docking site for the inner rod. (D) Contoured, longitudinal sections show the overall protein density distribution present in the base and the needle complex. (E) Key dimensions are given in Angstroms.*

purify sufficient amounts of the entire 'needle complex' and its precursor, the 'base', by a combination of detergent extraction, and size separation by velocity gradient centrifugation. Our biochemical analysis using quantitative amino acid analysis showed that the membrane associated base proteins are present in equimolar amounts (PrgH:PrgK:InvG = 1:1:1). On a structural level, this suggests that the three proteins are likely to share the same rotational symmetry of the cylindrically-shaped base. We were intrigued, however, to discover that the membrane-anchored base can adopt different sizes. A detailed structural analysis by three-dimensional electron cryomicroscopy and single-particle analysis finally revealed that several rotational symmetries or oligomeric states are present in the population of the 'needle complex' and the 'base'. Whether all of these complexes have a physiological role remains an open question.

In addition, our analysis identified a new structural component, the inner rod, which is located in the center of the needle complex (Figure 2). It (1) extends the secretion path from the base into the needle filament, and (2) serves also as an anchor to stably connect the needle filament into the base. During assembly, the inner rod and the needle filament are added as new structural components to the base. As a consequence, it must undergo large conformational rearrangements, which demonstrates the flexible but also stable property of the base. Functionally, this dynamic behavior is a crucial event during the assembly process, which reprograms the secretion machine such that it becomes competent for the secretion of virulence factors only after the growth of the needle filament is completed.

Although the design of the TTSS appears to be conceptually simple, structural characterization of the needle complex is at an early stage, leaving many questions unanswered: What nucleates the assembly of the TTSS? How dynamic is the entire assembly process? How does the export machinery interface with the needle complex and what determines the substrate specificity for protein secretion? We have just begun to address such questions, and we hope that by understanding the molecular mechanism of TTSS-mediated protein transport we may provide the basis for the development of novel therapeutic strategies to either inhibit its activity or modify the system for a targeted drug delivery.

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JAN-MICHAEL PETERS
Mitosis

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To pass the genome from one cell generation to the next, mitotic cells must package replicated DNA into chromosomes, attach the chromosomes to both poles of the mitotic spindle and then separate the chromosomes into their two sister chromatids. We are interested in understanding these processes at the molecular level.

Sister Chromatid Cohesion

Sister chromatids remain connected from S-phase until chromosomes are segregated in the subsequent mitosis or meiosis. This cohesion is required for repair of DNA double-strand breaks during G2 phase and for biorientation of chromosomes on the mitotic spindle in metaphase. Cohesion depends on cohesin, a multi-subunit protein complex that is loaded onto chromatin by the Scc2/Scc4 complex during telophase, and that establishes cohesion during S-phase. In vertebrates, the bulk of cohesin is removed from chromosome arms in prophase by a mechanism that depends on the cohesin-associated protein Wapl and in part on phosphorylation of the cohesin subunit SA2 by the mitotic kinase Plk1. At centromeres, cohesin is protected from this "prophase pathway" by the Sgo1 protein until all chromosomes have been attached to both poles of the spindle. At this stage, the protease separase is activated, cleaves centromeric cohesin and thereby initiates sister chromatid separation. We are interested in understanding how the interaction between cohesin and chromatin is regulated, and how cohesin establishes cohesion.

The Anaphase Promoting Complex/Cyclosome (APC/C)

The APC/C is a 1.5 MDa ubiquitin ligase complex that targets specific proteins for destruction by the 26S proteasome. These reactions are essential for progression through mitosis, meiosis and G1-phase of the cell cycle. APC/C is activated early in mitosis by phosphorylation and binding of the co-activator Cdc20, but its ability to ubiquitinate substrates is largely suppressed by the spindle assembly checkpoint until all chromosomes have become bioriented on the mitotic spindle. In metaphase, APC/C mediates the destruction of the separase inhibitor securin and of B-type cyclins and thereby allows activation of separase and inactivation of Cdk1, respectively. These reactions lead to the initiation of anaphase and to exit from mitosis. At the same time, Cdc20 is replaced by the co-activator Cdh1, which keeps APC/C active throughout G1. Cdc20 and Cdh1 are required for recruitment of substrates to the APC/C, but how substrates are ubiquitinated by the APC/C and how this process is controlled by the spindle assembly checkpoint is poorly understood. We are using biochemical approaches in human cells, Xenopus egg extracts and yeast to address these questions, and we are collaborating with crystallography and electron microscopy groups to analyze the structure of APC/C.

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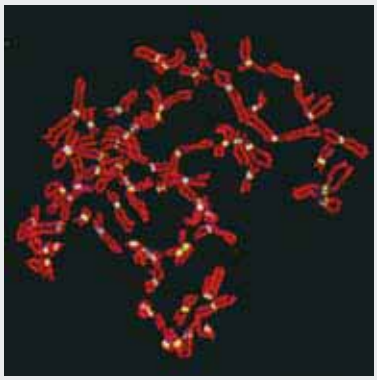

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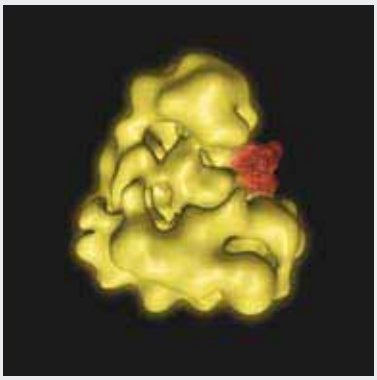

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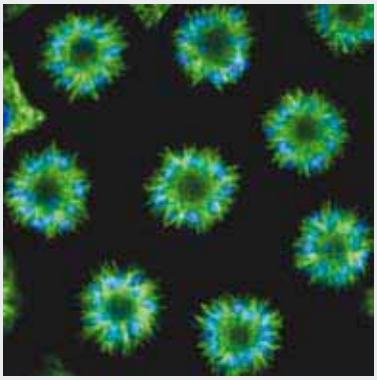

Figure 1: *Human mitotic chromosomes stained for condensin (red), cohesin (blue) and the centromere-specific histone CENP-A (green). Courtesy of Peter Lénárt.*

Figure 2: *A 3-D model of Xenopus APC/C bound to its co-activator, Cdh1. The model was obtained by angular reconstitution of negatively stained cryo-electron microscopy preparations. A WD40 domain that is found in Cdh1 has been modeled into the density that has been attributed to Cdh1 (colored in red). For details, see Kraft et al., Mol Cell, 2005; Dube et al., Mol. Cell, 2005. Courtesy of Holger Stark.*

Figure 3: *Human cells treated with the Plk1 inhibitor BI 2536 arrest with monopolar spindles (microtubules are stained in green and chromosomes in blue). Courtesy of Peter Lénárt.*

Mitotic Kinases

Entry into mitosis and virtually all steps during mitosis are controlled by a small set of protein kinases, such as Cdk1, Plk1 and Aurora A and B. We identified the small molecule, Hesperadin, as an inhibitor of Aurora B and found that this kinase is required for correcting syntely, a type of attachment in which both sister kinetochores of a chromosome become erroneously attached to one spindle pole. We discovered that Aurora B is also required for the spindle assembly checkpoint, for dissociation of heterochromatin protein 1 from centromeres and for association of the condensin I complex with mitotic chromosomes. In collaboration with Boehringer Ingelheim, we are using the small-molecule inhibitor BI 2536 to study functions of Plk1 in mitotic entry, spindle assembly and cytokinesis and to evaluate Plk1 as a potential drug target in cancer therapy.

MitoCheck

Although the central importance of protein kinases in controlling mitosis has long been recognized, little is known about their substrates and how phosphorylation regulates these substrates. To be able to address these questions in a systematic fashion, we have founded a consortium of eleven European institutes and companies who together have developed approaches to study mitosis in an Integrated Project that is funded through the 6th Framework Programme of the European Union. Major goals of this project, which is called MitoCheck, are to use genome-wide RNA interference screens to identify human proteins required for mitosis, to use mass spectrometry to systematically identify interaction partners and to map mitotic phosphorylation sites on these proteins, and to use small molecule inhibitors to identify substrates of Aurora B and Plk1.

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SIMON RUMPEL
Synaptic and Circuit Mechanisms of Memory Formation

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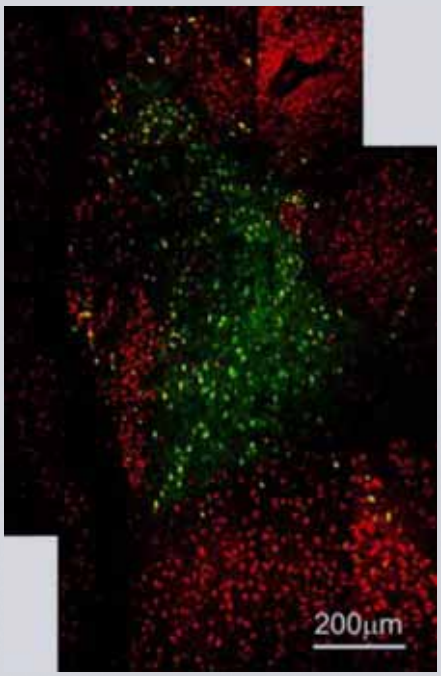

Our lab is interested in the development, function and plasticity of neuronal circuits. More specifically, we would like to learn how we are able to store memories over long periods of time. This is fundamental to the understanding of our minds: memories of past experiences shape our personalities and influence our current perception.

Trafficking of Synaptic Receptors during Memory Formation

Long-term storage of information about relevant experiences is essential for successful adaptation of human and animal behavior in a changing environment. A current model of memory formation suggests plastic adaptations in neuronal connections (synapses) caused by relevant experiences. The molecular mechanisms underlying synaptic plasticity have been mostly studied in cultured neurons. Synaptic plasticity is thought to be expressed, at least in part, through molecular changes that lead to addition or removal of synaptic AMPA receptors. In particular, synaptic addition of AMPA receptors containing the subunit GluR1 has been identified to be crucial for increases in synaptic strength. However, the processes actually happening in the brain during the formation of a memory trace are still poorly understood.

In order to delineate the role of particular candidate molecules, and to learn how memories are stored at the circuit level, we apply molecular tools in the behaving rodent in the context of memory-related behavior. Pavlovian conditioning is a simple and robust behavioral model of learning and memory formation, in which animals associate a tone with a mild foot-shock and subsequently "freeze" when presented with a tone alone. It has been previously shown that plasticity in the lateral amygdala plays an essential role in this behavior.

We recently tested the hypothesis that synaptic addition of GluR1-subunit containing AMPA-type glutamate receptors mediates an adaptation of synaptic strength in the lateral amygdala that is underlying the memory formation of the tone-shock association. We find that during Pavlovian conditioning about a third of amygdala neurons undergo synaptic plasticity by incorporation of GluR1-containing receptors. This suggests that the fear memory is encoded by many neurons and has a widely distributed nature. Surprisingly, we find that blocking synaptic delivery of GluR1-containing receptors in only 10-20% of amygdala neurons is sufficient to disrupt memory formation. This result indicates low redundancy in memory coding and suggests that successful memory retrieval requires participation of the vast majority of coding neurons.

Simon Rumpel / Group Leader (*since October*)

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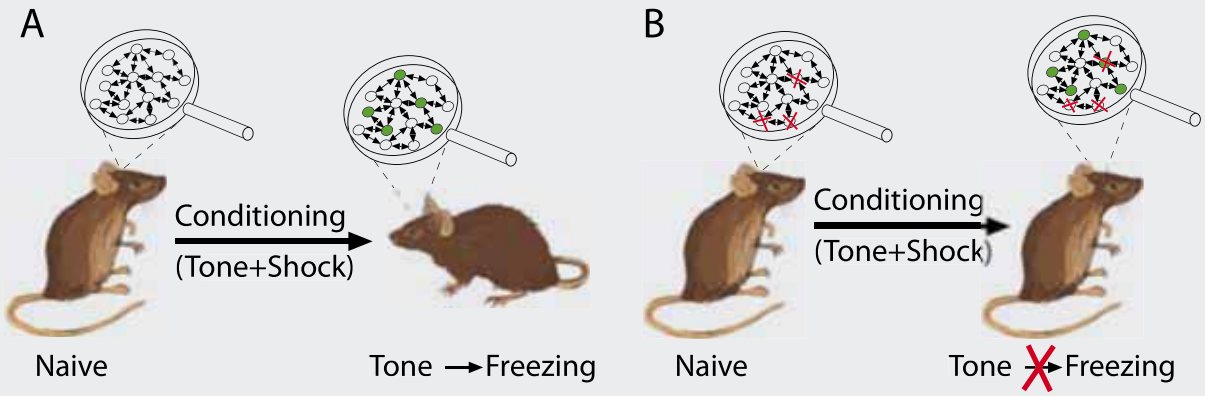

Figure 1: *Virus-mediated gene transfer in vivo allows specific manipulation of neuronal function during behavior. Neurons in the nucleus of the lateral amygdala express green fluorescent protein after in vivo infection. Red counterstain against neuronal marker NeuN.*

Figure 2: *Memory traces in the lateral amygdala appear to be distributed, but highly sensitive to perturbations of synaptic plasticity. (A) Rats show freezing behavior when presented to a tone that has been previously paired with a shock during a conditioning session. This behavior indicates that an association between the two stimuli was formed and successfully stored. About a third of neurons in the amygdala undergo plastic changes during conditioning and show synaptic incorporation of GluR1-type AMPA receptors (green circles). (B) Blocking GluR1-type AMPA receptor mediated plasticity in only 10-20% of neurons (red crosses) is sufficient to impair memory formation.*

Figure 3: *Chronic imaging of individual neurons and synapses. Two-photon laser scanning microscopy allows imaging of fluorescent neurons in the brain: Images of an individual neuronal process with synaptic contacts (so called spines, indicated by orange arrows) taken at different days. The connectivity of the neuron can be monitored by following the appearance, disappearance and size of synaptic spines.*

Peeking into the Brain

How can we continuously store novel memories in a given neuronal circuit without corrupting previously stored memories? In order to get a glimpse of the mechanisms how multiple memory traces are coordinated, we are currently applying chronic *in vivo* imaging techniques to the auditory cortex of mice. In addition to the amygdala, auditory areas of the cortex have been demonstrated to undergo learning-induced plasticity during auditory-cued fear conditioning. Two-photon laser scanning microscopy in transgenic animals expressing green fluorescent protein only in a small subset of cells allows revisiting the same neurons and even the same individual synapses day after day. This is truly remarkable since we estimate that the brain has about 10 trillion (10^{13}) synapses. In the future we plan to investigate the impact of auditory cued fear conditioning on the dynamics of a given set of synapses and neurons in the auditory cortex. We hope that this approach will help to open a door towards a series of novel experiments addressing information storage in living neuronal networks, a field of research that so far has been reserved mostly for theoretical neuroscientists.

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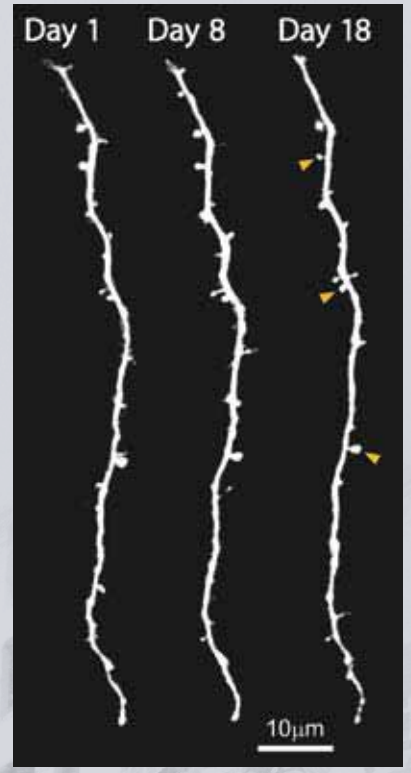

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PEGGY STOLT-BERGNER

Structural and Functional Analysis of Membrane Transporters

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Membrane proteins perform many vital cellular functions including energy metabolism, nutrient uptake, and signal transduction, yet comparatively little is known about their structure and the molecular mechanisms these proteins use to carry out these functions. We are attempting to increase the number of membrane protein structures, in particular structures of secondary transporters, through the identification of functionally relevant stable mutants that may be more amenable to crystallization. Functional studies on both the native and mutant transporters will also provide insights into the mechanism of substrate translocation across the membrane.

Membrane proteins make up 25-30% of most sequenced genomes and regulate many vital cellular processes such as energy metabolism, signal transduction, and transport of nutrients. Despite this, there are only approximately 100 known unique structures of membrane proteins, due to the challenges inherent in working with such hydrophobic proteins. Therefore, for many classes of membrane proteins there are few, if any, structural details available.

Until recently, secondary active transporters, which drive the transport of substrates against their concentration gradient by coupling this transport to an energetically favorable process, had not been structurally characterized. While recent work has begun to uncover some of the details underlying secondary transport mechanisms, there are still only a handful of individual structures of secondary transporters, and many important questions remain unresolved, such as how substrate and proton or ion translocation are coupled, and what conformational changes are necessary for transport to occur. In addition, due to the small number of available structures, it is likely that many transport mechanisms remain uncharacterized. Additional structures of diverse types of transporters and structures of different conformational states will most likely help to adress these questions.

Structural studies on membrane proteins are challenging primarily due to the instability of these proteins when outside of the native membrane environment. In addition, the conformational heterogeneity of membrane transporters can contribute to their instability (see Figure 1). One approach to facilitate the

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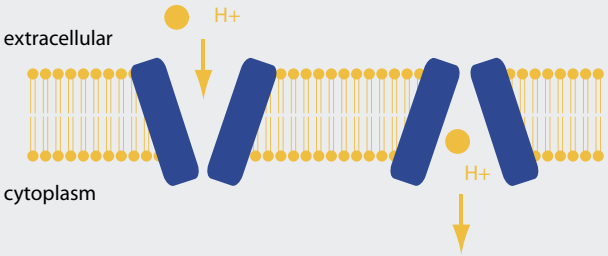

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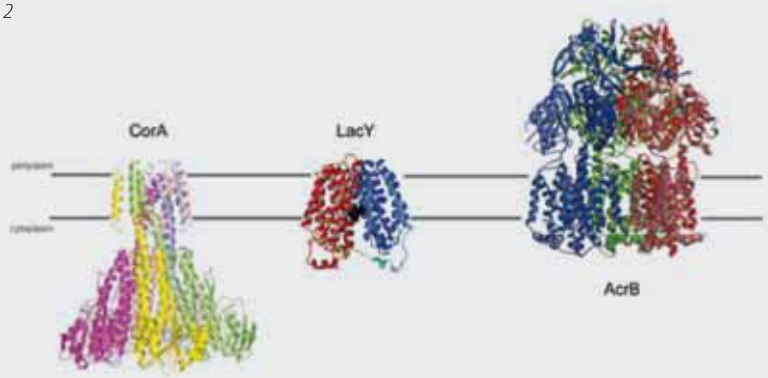

Figure 1: *The alternating access hypothesis of membrane transport. Secondary transporters have long been thought to function by alternating between at least two conformations. In one conformation the substrate binding site is open to the outside of the cell, and upon substrate binding, the protein undergoes conformational changes that open the substrate binding cavity to the inside of the cell. While there is some structural evidence to support this model, it does not apply to all transporters, as alternative transport mechanisms have also been characterized (see Figure 2).*

Figure 2: *Current transporter structures reveal diverse types of transport mechanisms. Recent structural work has uncovered evidence for multiple transport mechanisms. The E. coli lactose permease LacY adopts the alternating access model, which most likely involves large conformational changes (Abramson et. al., 2003 Science 301:610; figure adapted from Abramson et. al., 2004 Curr Opin Struc Biol 14:413). In contrast, the pentameric structure of the CorA divalent metal transporter from T. maritima suggests that its mechanism of transport is similar to that of a gated channel (Lunin et al 2006 Nature 440:833; Eshaghi et al 2006 Science 313:354; figure adapted from Eshaghi et al). The trimeric E. coli multidrug efflux pump AcrB utilizes an alternating binding site mechanism, which involves more subtle conformational changes (Murakami et al 2006 Nature 443:173; Seeger et al Science 313:1295; figure adapted from Murakami et al 2002 Nature 419:587). These diverse structures and mechanisms emphasize the need for structural characterization of a larger number of transporters, and for structures in different conformational states in order to generate deeper insights into these and other as yet uncharacterized transport mechanisms.*

crystallization of transporters, and membrane proteins in general, is to identify more stable forms of the protein of interest. This approach has already proven successful for transporters; LacY, the lactose permease from *E. coli*, was first successfully crystallized using a mutant transporter that shows very little transport activity but is still able to bind its substrate (Abramson et. al., 2003 Science 301:610).

Given the potential utility of this approach in increasing the number of transporter structures, we are interested in systematically identifying mutants that will prove amenable to crystallization, and thereby help produce novel structures as well as structures in different conformations. We are attempting to screen a library of secondary transporter mutants in the yeast *Saccharomyces cerevisiae* to identify more thermostable or conformationally stable mutants for crystallization trials, as well as loss-of-function mutants to aid in the identification of functional residues. Stable mutant proteins identified through this process will be over-produced for crystallization trials, and will be further characterized using cell-based and *in vitro* assays.

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ERWIN WAGNER
Gene Function in Mammalian Development and Disease

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The major focus of our studies is to analyze gene function in normal and pathological conditions, e.g. in tumor development, using the mouse as a model organism. Specifically, the functions of AP-1 in regulating cell proliferation, differentiation and cell death are investigated. Our studies revealed that the AP-1 proteins Fos and Jun play pivotal roles in bone, liver, heart, skin, hematopoietic and neuronal development.

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[3] DFG grant; since July 2004,
[4] EU-Anabonos; since January 2005,
[5] EMBO and Marie Curie fellowship,
[6] RTN-EU-grant,
[7] FWF-grant until January 2006,
[8] FWF-grant since April 2006,
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Fos/AP-1 - Functions in Bone Development

Fos proteins are key regulators of bone development. Transgenic mice over-expressing c-fos develop osteoblastic bone tumors, whereas mice lacking c-Fos are osteopetrotic and lack bone-resorbing osteoclasts (Figure 1). The Fos-related protein Fra-1 is essential for mouse placental development, whereas transgenic mice over-expressing Fra-1 develop an osteoblastic bone disease termed osteosclerosis. We generated conditional alleles of c-*fos* and *fra-1* to investigate how c-Fos and Fra-1 control osteoblast and osteoclast differentiation. The embryonic lethality of fra-1 knock-out mice was rescued by a conditional allele of fra-1 and the mutant mice were viable but developed osteopenia, a low bone mass disease. Recently, we analyzed the properties of the Fos-related protein Fra-2 using loss and gain of function approaches (Figure 2). We found that Fra-2 is essential for postnatal mouse development and maintenance of proper bone mass. Moreover, transgenic mice over-expressing Fra-2 have increased bone mass and develop pulmonary fibrosis. We are currently investigating the underlying molecular mechanisms leading to the bone and lung disease.
We have addressed the relevance of post-translational modifications of c-Fos in bone development and pathogenesis. The ERK-dependent kinase RSK2 can phosphorylate c-Fos on serines 362 and 374, and we were able to show that RSK2 kinase is essential for the development of c-Fos-dependent osteosarcomas *in vivo* (Figure 1). To further study c-Fos phosphorylation *in vivo*, c-Fos mutant knock-in mice were generated in which the endogenous wild-type c-*fos* allele was replaced by a point mutant allele with serines 362 and 374 mutated to alanines (FosAA). Recent results show that c-Fos C-terminal phosphorylation is dispensable for skeletogenesis but required for osteoclastogenesis *in vivo* and for normal bone homeostasis and tumor formation in adult mice.

Tumor Suppression by JunB and Fos

JunB is a transcriptional activator of the cyclin-dependent kinase inhibitor p16/INK4a and functions as a negative regulator of cell proliferation in fibroblasts. Using conditional gene ablation we could show that JunB acts as a tumor suppressor *in vivo*. JunB absence in the myeloid lineage led to a transplantable myelo-proliferative stem cell disease resembling human chronic myeloid leukemia.
Initially identified as a *bona fide* oncogene, the Fos protein was found to have an unexpected function as a tumor suppressor in the muscle lineage:

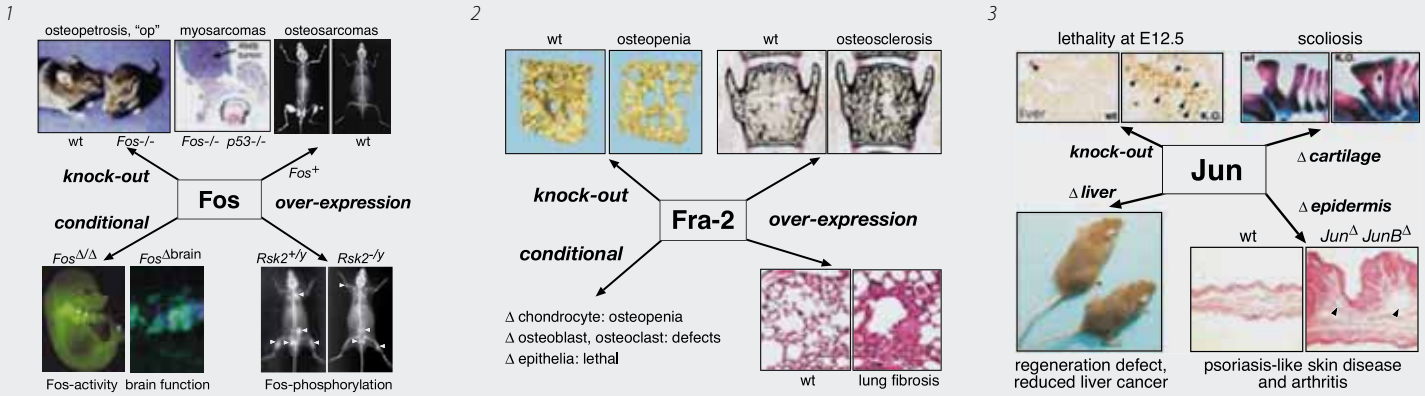

Figure 1-3: Functional analysis of Fos (1), Fra-2 (2) and c-Jun in development and disease.

loss of both Fos and p53 resulted in the specific formation of rhabdomyosarcomas with a frequency greater than 90% (Figure 1). Re-expression of Fos in double mutant muscle tumor-derived cell lines induced apoptosis, indicative of a novel mechanism of tumor suppression by Fos.

Jun/AP-1 - Role in Proliferation, Differentiation and Apoptosis

Using conditional mutagenesis, knock-in strategies and transgenic rescue experiments, we have demonstrated that Jun is essential for liver regeneration and is required as a survival factor during liver tumor development (Figure 3). We were able to show that a p53/p21 and p38-MAPK-dependent pathway is operating to coordinate efficient liver regeneration. Analogous genetic strategies are employed to investigate the functions of Jun/AP-1 proteins in liver cancer and inflammatory disease, such as hepatitis and colitis-associated colorectal cancer. In these model systems, the role of AP-1 proteins in inflammatory cells as well as in intestinal epithelial cells is under study.

Deletion of Jun in the epidermis of the skin causes an eye closure defect and affects proliferation of keratinocytes *in vivo* and skin tumor development *in vivo* likely through down-regulation of HB-EGF and EGFR. When JunB was inactivated in the epidermis, mice are born healthy, but develop a multi-organ-disease likely caused by deregulated cytokine expression from keratinocyte-derived G-CSF and IL-6. In addition, we are able to demonstrate in patients´ samples and employing an inducible mouse model that downregulation of Jun and JunB proteins in keratinocytes can cause a psoriasis-like disease, which is a common chronic disease affecting skin and joints. Interestingly, when Jun and JunB were inactivated in the epidermis in a constitutive manner, mutant pups died due to a cytokine storm, which involves deregulation of TNF-TIMP3 expression.

VEGF, VEGF-R2/Flk-1 and EGF-R and p38

Conditional alleles of VEGF, Flk-1 and EGF-R are employed to study the role of VEGF and Flk-1 in the development of endothelial and hematopoietic stem cells. In collaboration with Erwin Tschachler and Maria Sibilia from the Department of Dermatology, Medical University of Vienna, we are investigating the functional importance of VEGF-A in skin biology and the role of EGF-R in normal and skin tumor development. In addition, we have employed conditional alleles of the MAPK p38, obtained from BI Ridgefield, to study their functions in postnatal development, in muscle cell biology and in liver cancer.

STEFAN WESTERMANN
Molecular Mechanisms of Kinetochore Function

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Chromosome segregation requires a physical connection between the centromeres of chromosomes and the microtubules of the mitotic spindle. One of the most fascinating questions of mitosis is how the kinetochore, the protein complex that assembles on centromeric DNA, mediates the attachment of chromosomes to highly dynamic microtubule plus ends and how it creates sufficient force to power sister chromatid segregation during anaphase. We are studying kinetochore function in the budding yeast Saccharomyces cerevisiae using a combination of biochemistry, yeast cell biology and in vitro assays with dynamic microtubules to gain insights into the molecular mechanisms of chromosome segregation.

Organization of the Budding Yeast Kinetochore

Despite the small size of its centromeric DNA, the budding yeast kinetochore is a complex macromolecular structure that consists of more than 65 proteins. These proteins do not just form a simple attachment site, but they assemble into a molecular machine that controls and powers chromosome movement, monitors the state of attachment and tension across sister chromatids and signals to the mitotic checkpoint. Systematic tandem affinity purifications and mass spectrometric analysis of kinetochore proteins have identified the budding yeast kinetochore as a hierarchical assembly of multi-protein complexes (Figure 1). Many of these complexes have homologs in higher eukaryotes, suggesting that fundamental aspects of kinetochore organization are conserved throughout evolution. A challenge for the future is to understand the precise molecular functions of individual kinetochore complexes, to reassemble them into a functional kinetochore *in vitro* and to analyze how their function is regulated by posttranslational modifications.

Formation of a Dynamic Kinetochore Interface

We have started to reconstitute individual kinetochore complexes by co-expressing their subunits in insect cells or bacteria to analyze their structures, biochemical activities and interactions. Electron microscopy has revealed that the 10-protein Dam1 complex, a key microtubule-binding element of the budding yeast kinetochore, oligomerizes into a 16-fold ring around a microtubule *in vitro* (Figure 2). Importantly, this protein ring does not have a fixed binding site on the mircrotubule, but is able to slide laterally along the lattice, allowing the formation of a dynamic attachment site for the kinetochore.
Many questions about the kinetochore ring complex remain unanswered: How is the assembly and dynamics of the ring regulated? How does it connect to the other multi-protein complexes of the kinetochore? How does it co-operate with motors and plus-end tracking proteins to form a functional kinetochore attachment site at the microtubule plus-end?

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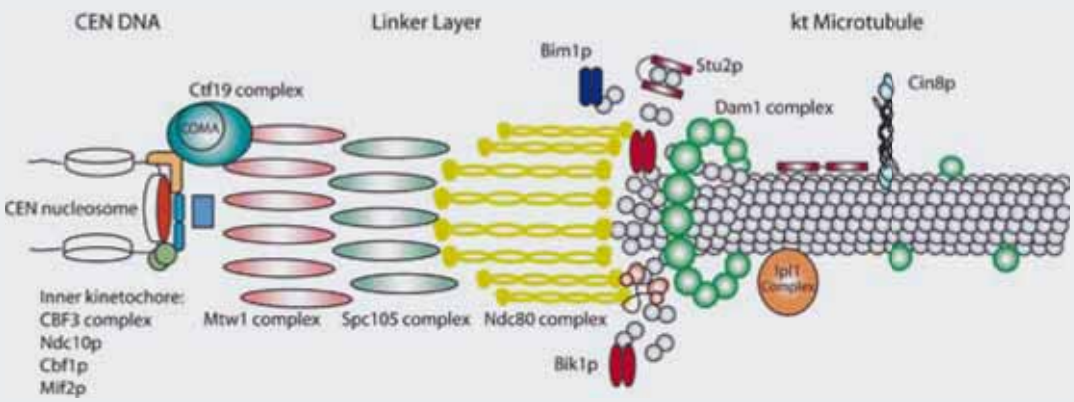

Figure 1: *Schematic illustration of the budding yeast kinetochore. The kinetochore is an assembly of multi-protein complexes that connects centromeric DNA to the plus-ends of spindle microtubules. The Ipl1 complex phosphorylates multiple components to regulate microtubule-kinetochore interactions.*

Figure 2: *Electron microscopy reveals that the 10-protein Dam1 complex oligomerizes into a ring around taxol-stabilized microtubules in vitro.*

Figure 3: *The Dam1 ring complex stays attached to the end of a disassembling microtubule. (A) Sketch of the microscopy assay using Rhodamine-Tubulin and Alexa488-labeled Dam1 rings. (B) Stills from a movie showing that the Dam1 ring (green, arrowhead) follows the end of the depolymerizing microtubule. (C) Principle of a novel biological motor: Upon GTP-hydrolysis, microtubule protofilaments curve and push the Dam1 ring over the microtubule lattice. This mechanism can connect the kinetochore to a depolymerizing microtubule.*

Mechanisms of Force Generation

One of the key features of mitosis is that the chromosomes are able to hold on to the ends of disassembling kinetochore microtubules during Anaphase A and harvest the force generated by microtubule depolymerization for their transport towards the spindle poles. The assembly of the Dam1 ring complex can provide an elegant mechanical solution to the attachment problem. Using Dam1 rings labeled with a fluorescent dye and in vitro microscopy assay, we could directly visualize that the ring stays attached to the end of a disassembling microtubule and slides over the lattice towards the minus end of the shrinking polymer (Figure 3). This constitutes a new mechanism for microtubule-based motility that uses the energy of GTP hydrolysis by the microtubule and converts it into a processive movement. We will continue to develop microscopy assays with dynamic microtubules to uncover further aspects of kinetochore motility.

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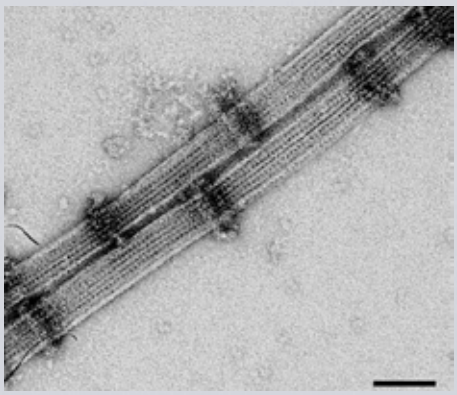

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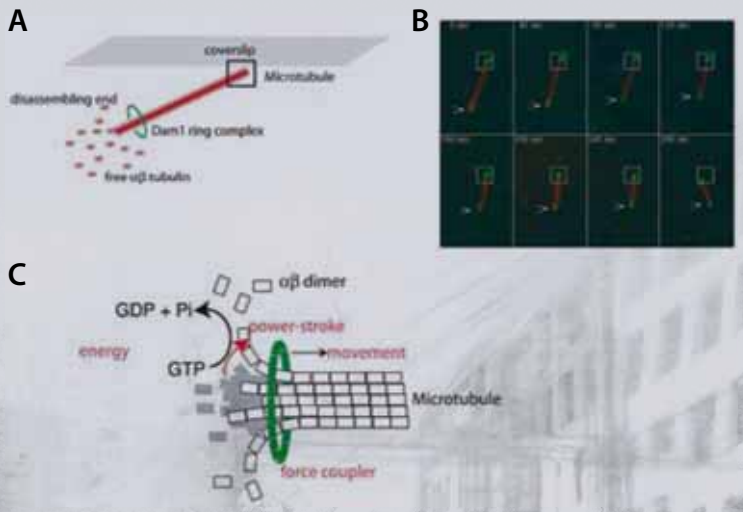

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ANTON WUTZ
Mammalian X-Chromosome Inactivation

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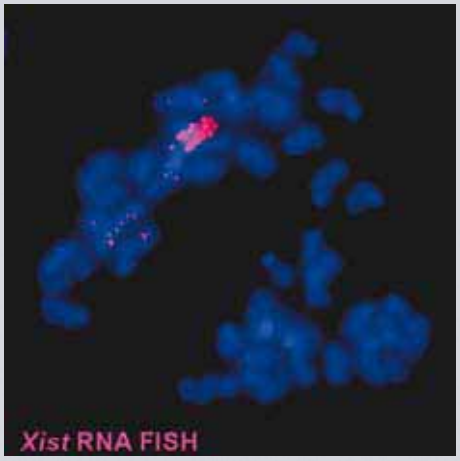

For successful development, the information stored in the genome needs to be precisely regulated. During differentiation, each individual cell uses an ever-changing repertoire of epigenetic mechanisms to achieve proper control of gene expression. Our research focuses on the regulated formation of heterochromatin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

X inactivation is the process that mammals use to compensate for the dosage difference in X-linked genes between the sexes. This is achieved by transcriptional silencing of one of the two X-chromosomes in female cells. The long non-coding *Xist* RNA is critical for initiation of this silencing process and associates physically along the entire chromosome (Figure 1).

The Function of Polycomb Group Proteins at the Initiation of X-Inactivation

Polycomb group (PcG) complexes are recruited by *Xist* at an early stage in X inactivation and mediate chromosome-wide modifications of histones. We have shown that recruitment of Polycomb repressor complex 1 (PRC1) and PRC2 is strictly dependent on *Xist* RNA at all stages of differentiation, and PRC1 can be recruited by *Xist* independent of PRC2 (Schöftner et al., 2006). Notably, we find that *Xist* can initiate chromosome-wide silencing in ES cells lacking PRC2 activity by a mutation in *Eed*. Thus, PRC2 is not essential for X inactivation in embryonic cells. Eed is essential for recruitment of the PRC1 proteins Mph1 and Mph2 but not Ring1b by *Xist*. Thus, PRC1 proteins are partially recruited in the absence of PRC2 and mediate ubiquitination of histone H2A. *Xist* expression early in ES cell differentiation establishes a chromosomal memory, which allows efficient H2A ubiquitination in differentiated cells and is independent of silencing and PRC2. Thus, *Xist* recruits PRC1 components by both PRC2 dependent and independent modes and in the absence of PRC2 function is sufficient for the establishment of Polycomb-based memory systems in X inactivation. Since PRC1 and PRC2 are essential for embryogenesis and are independently recruited by *Xist*, we propose that they might act redundantly during X inactivation (Figure 2). Future experiments aim for the establishment and characterization of ES cell lines deficient in PRC1 and PRC2 function.

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Turning off Chromosomes by *Xist* during Development

Xist is the trigger for X inactivation early in embryogenesis. In differentiated cells, *Xist* becomes dispensable for the maintenance of the inactive X, and its function for initiation of silencing is lost. Thus, initiation of silencing is restricted to a specific time interval at the onset of cellular differentiation. How *Xist* mediates gene repression remains an open question. We use an inducible *Xist* allele in mice

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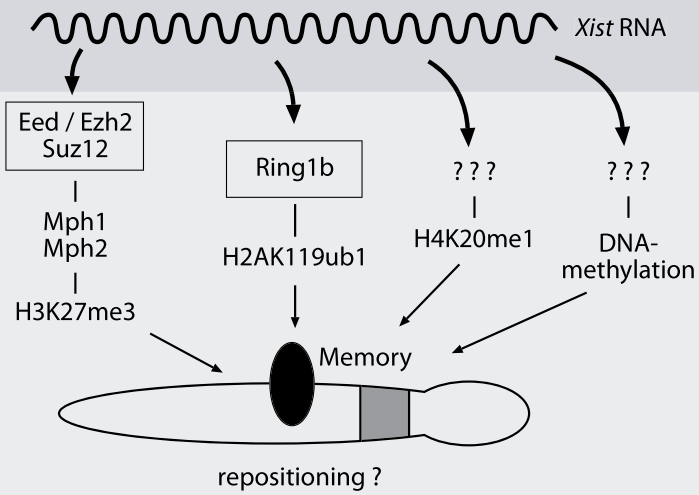


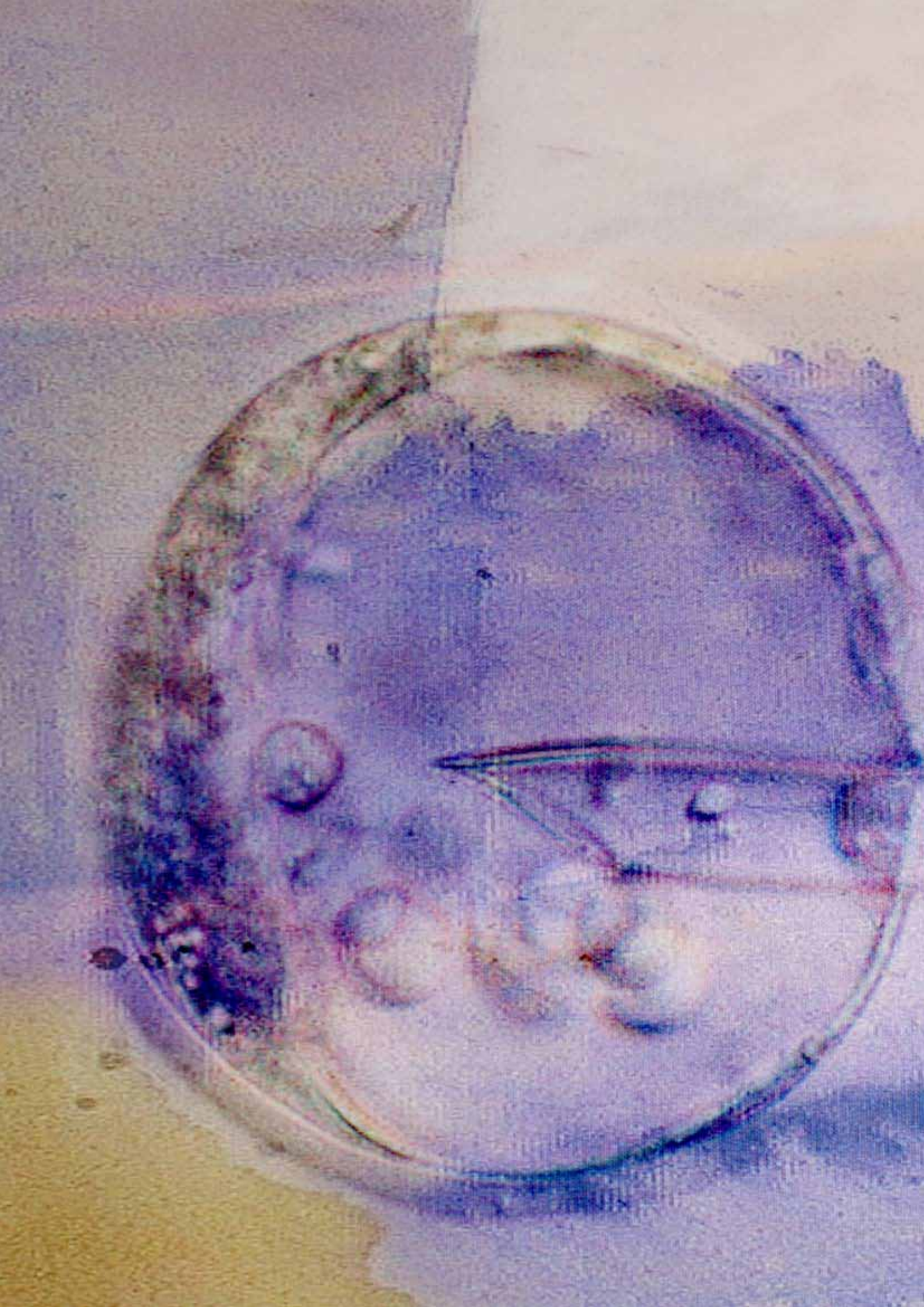
Figure 1: *Xist RNA association with chromatin revealed by RNA FISH. Mouse chromosome spreads were prepared from ES cells expressing Xist from a transgene on chromosome 11 and hybridised with a fluorescent probe detecting Xist RNA (red). DNA was counter stained with DAPI (blue).*

Figure 2: *Recruitment of Polycomb group proteins and histone modifications by Xist. Xist expression induces gene silencing and recruitment of chromatin modifying complexes in parallel pathways. The perceived mode of recruitment is indicated in the scheme.*

to identify cells in which *Xist* can cause chromosome-wide silencing. Our results indicate that the potential of *Xist* to initiate silencing is lost during embryogenesis in a gradual and cell type-specific manner (Savarese et al., 2006). In adult male mice, ectopic expression of *Xist* causes an anaemia that can eventually lead to lethality. *Xist* has the ability to initiate silencing in immature haematopoietic precursor cells. In contrast, haematopoietic stem cells and mature blood cells are unable to initiate ectopic X inactivation. This indicates that critical pathways for silencing are transiently activated in haematopoietic differentiation. *Xist*-responsive cell types in normal female mice show a change of chromatin marks on the Xi. However, dosage compensation is maintained throughout haematopoiesis. Therefore, *Xist* can initiate silencing in precursors with concomitant maintenance of dosage compensation. This suggests that *Xist* can function in cells of the adult organism and points to epigenetic differences among specific stages of the haematopoietic differentiation cascade. Further work is directed towards understanding the molecular basis for this epigenetic difference.

Identification of Proteins that Interact with *Xist* RNA in Chromosomal Silencing

To gain insight into the molecular mechanism by which *Xist* causes transcriptional repression, we focus on the isolation of proteins that interact with the repeat A of *Xist* RNA, a crucial element for initiation of silencing. In collaboration with Karl Mechtler from the IMP protein sequencing facility we have identified proteins from nuclear extracts that specifically interact with repeat A RNA. Ongoing experiments focus on the functional analysis of candidate proteins and the identification of a potential pathway for *Xist* mediated transcriptional silencing.
Functional studies of X inactivation will 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 determine its specific nuclear localisation. It is expected that similar interactions underlie the regulation of other genes – however, with less dramatic consequences. Regulation of chromatin by non-coding RNAs is the focus of contemporary epigenetic research and might pinpoint a general process by which transcription feeds back onto the chromatin structure.



BIOOPTICS DEPARTMENT

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[1] *until July,*
[2] *February – July*

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

Current Research Activities

During the past year, one focus of our department was the implementation of multiplexed bead-assays for the quantification of various analytes using a BioPlex System. Even though a wide variety of kits are commercially available, standard methods for the generation of bead assays for more "exotic" analytes have been established.

In order to comply with increased demand by our users, the micro-array service now provides complete service including amplification and labeling, hybridization, image analysis and simple statistical analysis. Besides the convenience for the users the main reason to do so is to increase the robustness, significance and reproducibility of the obtained results.

To achieve these goals, we have established standard protocols for all steps involved with a strong focus on quality control of every step and the use of an automated hybridization station. Optimization of these procedures also allowed a reduction in overall costs, making it possible to routinely perform at least three technical replicates per sample. Comparative analyses with other platforms including Affymetrix revealed a very high correlation of the results obtained

As the production of the cDNA-arrays has been optimized for semi-automated image analysis, this step can now also be performed with minimal human interaction. The data generated by the image analysis is then fed into a processing pipeline where normalization and initial statistical analysis has been automated using modules from the BioConductor package adapted to the experimental designs currently applied. In a collaboration with Arndt von Haeseler (CIBIV, University of Vienna), more elaborate statistical tools will be implemented.

The supply of available cDNAs has also significantly increased by the acquisition and processing of 100,000 mouse cDNA clones of high quality from the RIKEN institute. Currently, the cDNAs are spotted on a set of arrays, which will be the basis of the future standard set of arrays. Together with very specialized arrays resulting from SSH-derived cDNA-libraries, we aim to cover most of the mouse transcriptome in the near future.

ELECTRON MICROSCOPY

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Günter Resch / Head of Facility

Nicole Aichinger[1] /Technical Assistant

[1]*until November 2006*

The Electron Microscopy Facility, established in the course of this year, offers a wide variety of preparation techniques for visualisation of tissues, cells and purified molecules by transmission electron microscopy, as well as support with microscopy and data management. In addition to the routine equipment up and running, preparations to set up a cryo-capable high-end microscope in 2007 are in progress.

Along with the move into the Academy Laboratory Building at the beginning of 2006, the work to establish an Electron Microscopy Facility at the IMP-IMBA Research Center entered a new phase.

A major part of starting up the facility was setting up and testing a good deal of specialised preparation equipment, including ultramicrotomes, high vacuum evaporators, glow discharge units, a freeze substitution device, etc. Routines for performing diverse preparation techniques were established, including: chemical and physical fixation, conventional resin embedding, freeze substitution, ultrathin sectioning, production of support films, negative staining, rotary shadowing of sprayed molecules, and others. Most of these approaches are now routinely used by both IMP and IMBA researchers as well as within external collaborations. Depending on future developments and the focus of the demand from the institutes, more preparation procedures will be made available.

With the long awaited installation of the FEI Morgagni transmission electron microscope in July, the major equipment for service purposes was completed. This robust and easy to use 100 kV instrument is tailored for routine needs in the multiuser environment of a facility. For suitable documentation of large fields as well as high resolution imaging, it is equipped with a 11 megapixel CCD camera. Immediately following its installation, the microscope was heavily booked, reflecting a local demand for electron microscopy in both institutes.

To support users with data management, a web-based project-oriented database system named MIMAS was established. All electron micrographs including metadata can be stored on and accessed from this database on a user restricted basis. It is now running in an early version and continuously being developed into a more stable and versatile platform, in close collaboration with users to meet their needs.

In parallel with these daily activities, the EM Service is overseeing the pre-installation of the FEI TF30 Polara. This 300 kV transmission electron microscope, equipped with the most advanced imaging systems, was funded by a Vienna Spot of Excellence grant to the Marlovits, Peters, Small and EM Service groups. It is scheduled to be delivered to site at the end of 2006 and planned to commence operations in the course of 2007. Primarily, it will be used for cryo-electron microscopy of molecules and cells, electron tomography, as well as analytical applications.

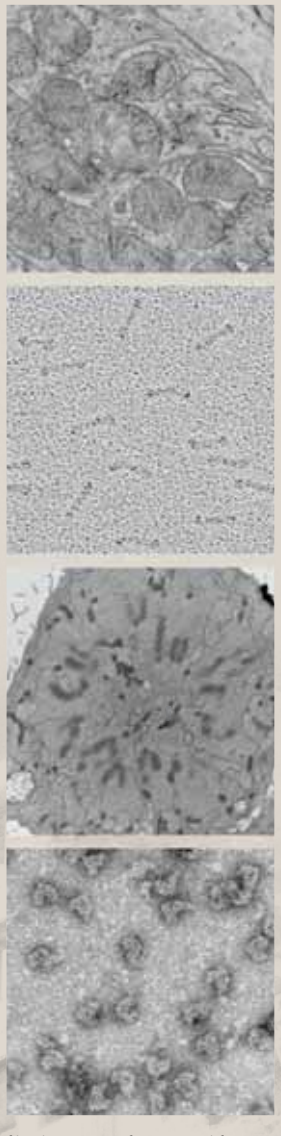

Figure 1: Techniques applied in the Electron Microscopy Service, from top to bottom : Ultrathin section of high pressure frozen and freeze substituted kidney tissue, glycerol sprayed and rotary shadowed α-actinin molecules, mitotic HeLa cell after flat embedding and sectioning (micrograph by Peter Lénárt), and negatively stained APC molecules.

BIOINFORMATICS SUPPORT

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Maria Novatchkova / Bioinformatics Support

The Bioinformatics Support unit is part of the Bioinformatics Research group of Frank Eisenhaber, and assists research groups in molecular biology-related fields, providing infrastructure, sequence-analytic services, support and education in bioinformatics.

The IMP-IMBA Bioinformatics support unit has its main expertise in the field of sequence-analysis. Typical tasks include the functional and structural characterization of proteins and genomic regions, using methods such as pattern matching, complexity analysis and homology searches.

Often problems arise from functional genomics or high-throughput biological datasets, for which we develop computational and mathematical solutions able to cope with the high load and memory requirements.

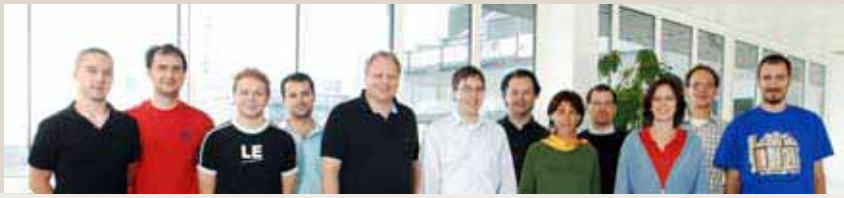
Software and Database Development

In order to efficiently handle recurring tasks, we also engage in custom software and database development. This year we have publicly released DOUTfinder, a software tool dedicated to the improved identification of known domains in protein sequences. True similarities to functionally described domains can easily be missed if they are reported as false negatives below recommended significance thresholds (see Figure 1). DOUTfinder is used to suggest biologically meaningful twilight zone domain similarities by providing a homology-backed procedure for filtering of relevant subthreshold hits.

Training

The IMP ANNOTATOR is an in-house facility providing a user-friendly web interface to integrate complex sequence-analytic tasks. We provide hands-on training courses on the use of the ANNOTATOR system, where participants learn the basis and limitations of sequence analysis.

Figure 1: *The tri-dimensional structure of a single CH domain (1H67) can serve as a model for understanding the organization of the N-terminal globular region in NuMA proteins. The similarity between the NuMA N-terminus and the CH domain is reported as a false negative and sub-significant hit in sequence similarity searches, but can be supported by DOUTfinder and structural similarities.*



PROTEIN CHEMISTRY FACILITY

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The IMP-IMBA Protein Chemistry Facility performs a large variety of mass spectrometry experiments, including identification of proteins by peptide sequencing and characterization of post-translational modifications (PTMs), such as phosphorylation. In addition, we are developing new methods for the quantification of PTMs. Finally, our facility specializes in peptide synthesis and antibody purification.

Karl Mechtler / Head of Facility

Goran Mitulovic / Postdoc
Karin Grosstessner-Hain / PhD Student
Nedim Mujezinovic / PhD Student
Otto Hudecz / Technician
Richard Imre / Technician
Gabriela Krssakova / Technician
Mathias Madalinski / Technician
Michael Mazanek / Technician
Michael Schutzbier / Technician
Ines Steinmacher / Technician
Christoph Stingl / Technician

Improvement in Phosphorylation Analysis

Protein phosphorylation is the most important reversible post-translational modification. Thus, analysis of phosphorylated proteins and identification of the phosphorylation sites help us to understand their biological functions.

A. Analysis of protein complexes
We have developed a new offline chromatographic approach for the selective enrichment of phosphorylated peptides that is directly compatible with subsequent analysis by online nano electrospray ionization tandem mass spectrometry (ESI-MS/MS). Here, a titanium dioxide-packed pipette tip is used as a phosphopeptide trap for offline first dimension separation step in a two-dimensional chromatography system. This is followed by online nano reversed-phase high-performance liquid chromatography.

B. Enrichment of phosphopeptides from complex mixtures
Immobilized Metal-ion Affinity Chromatography (IMAC) was established for enrichment of phosphopeptides from complex mixtures. This technique, which is based on the affinity of negatively-charged phosphate groups for positively-charged metal ions (Fe^{3+}), immobilized on a chromatographic support.

C. Application of Different Fragmentation Techniques for the Analysis of Phosphopeptides using an Ion Trap - FTICR Mass Spectrometer
Electron capture dissociation (ECD) is a complementary technique for the fragmentation of peptides and proteins in mass spectrometry in addition to the commonly used collisionally activated dissociation (CAD). ECD has been shown to be applicable for the efficient sequencing of peptides and proteins, and has been proven especially valuable for mapping labile PTMs, such as phosphorylation sites.

The Christian Doppler Laboratory for Proteome Analysis. Together with Prof. Gustav Ammerer from the Max F. Perutz Laboratories we have established a project for the quantitative analysis of proteins based on mass spectrometry. Our goal is to study the composition of multi-protein complexes and their associated partners.

Peptide Synthesis and Antibody Purification. We synthesize about 350 peptides per year, including an increasing number of branched peptides containing acetylated, phosphorylated or methylated amino acid residues. We have developed procedures for affinity-purification of antibodies, including optimised elution under mild conditions.

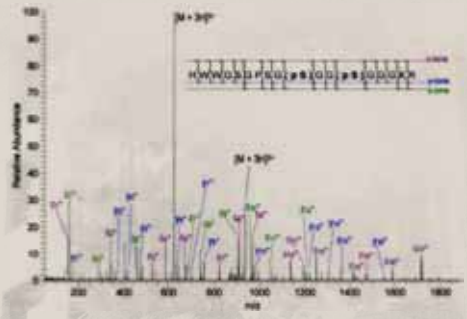

Figure 1: ECD product ion spectrum for the fragmentation of a triply-charged peptide $[M+3H]^{3+}$, at m/z = 625.2) reveals the positions of two phosphoserine residues.

HISTOLOGY DEPARTMENT

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Vukoslav Komnenovic / Head of Facility

Laura Göderle / Technician

The Histology Service Department offers state-of-the art sectioning, histology and immuno-histochemistry services to all IMBA and IMP scientists. In addition, we offer training and support for researchers in new technologies.

Histology Services

The histology services include the embedding of tissues, assistance with the sectioning of paraffin and cryo-preserved tissues, and preparation of slides for standard H&E stains, as well as specialized stainings such as PAS, Alcian blue, Cab, Gomeri, MayGruenwald-Giemsa and van Kossa stains for human, mouse, Xenopus and Drosophila studies. With these services, we are able to offer support to get quick results.

Sectioning of Paraffin and Frozen Tissues

In our group we have developed a high throughput method to cut paraffin and frozen tissues. Using this method, we could increase the quality and also the quantity of services.

Immunohistochemistry

The Histology Service Department also provides automated preparation and processing facilities for standardized immuno-histochemistry, in situ hybridization, FISH analysis, DNA microarray and tissues microarray applications. Various antibodies have been characterized for optimized in situ signaling studies. Standard antibodies such as apc, cd (several markers), l-ad, gfp, gfap, c-fos, c-jun, junB, fra1,2, jun-D, ki67, smad3, brdu, egf, egfr, H3K9me tri meth, H4K20me3 tri meth, cl. caspase3, caspase7, procatepsiK are available.
In addition, the Histology Service Department administrates legal regulatory affairs such as the record-keeping and documentation of experiments in accordance with the Austrian Histolaboratories guidelines (www.mta-labor.info).

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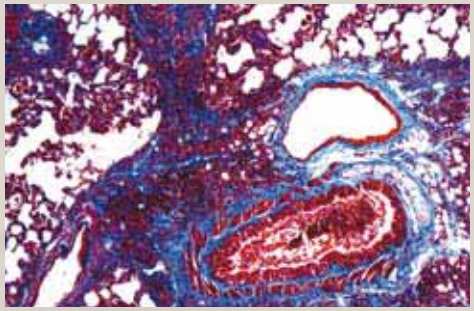

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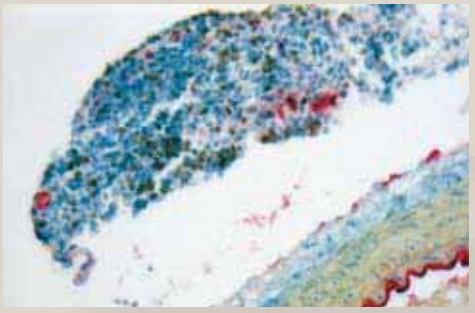

Figure 1: Trichrom blue staining. The picture shows 4 different signals, light blue for Lung fibrosis, red for Mast cells and Neutrophils, pink for Myeloiud cells and dark blue for the Nuclei.

Figure 2: Double Staining with Ki67 and von Willebrand Factor. Ki67 turnes out to be brown whereas vWF shows a red signal. The counter staining was done with Hematoxylin (blue).

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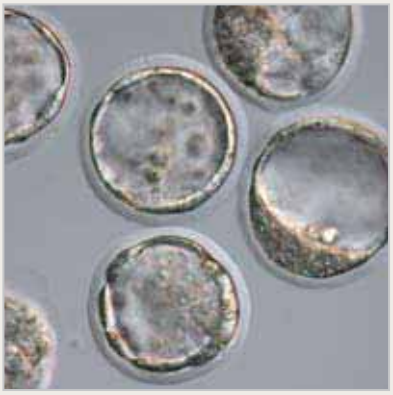

ANIMAL HOUSE

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MOUSE SERVICE

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The Animal House *group provides husbandry of animals and services for the various research groups at the IMP and IMBA.*

Animal House

Andreas Bichl / Head, Veterinarian
Erwin F. Wagner / Scientific Coordinator
Norma Howells / Consultant

Husbandry

The husbandry is divided into two main areas containing the following species: mice and *Xenopus*. The larger 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 the 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 the ordering of mice from external breeding companies, and the 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.

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 Mouse Service Department services are shared by the IMP and IMBA.*

Mouse Service

Hans-Christian Theussl / Head of Facility
Jacek Wojciechowski / Technician

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 and IMBA staff.
About 50 different ES cell clones and several DNA constructs are being 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.

Figure 1: *Injection of embryonic stem cells into mouse blastocyst.*

Figure 2: *Mouse blastocysts.*

SERVICE DEPARTMENT

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Gotthold Schaffner / Scientist

Ivan Botto / Technician
Markus Hohl / Technician
Shahryar Tagybeeglu / Technician
Gabriele Botto / Technician Media Kitchen
Christa Detz-Jaderny / Technician Media Kitchen
Ulrike Windholz / Technician Media Kitchen
Franziska Stransky / Technician Fly Food Preparation
Anna Windholz / Technician Fly Food Preparation
Oliver Botto / Help Fly Food Preparation
Thomas Haydn / Help Fly Food Preparation

The Service Department offers a variety of high-quality and rapid services to IMP and IMBA scientists. The majority of our effort involves DNA sequencing, fly food production and preparation of various media and solutions.

Our Media Kitchen and Fly Food staff prepares substantial quantities of reagent quality solutions and media for cell culture, flies (approximately 1,200,000 bottles and tubes per year) and worms. We moved this year to the new IMBA building, where we have more space to create better and convenient working conditions for preparing fly food and all other products.
We also prepare many selected reagents such as DNA molecular-weight markers, enzymes, a variety of transformation-competent *E. coli* strains, and we maintain a stock of cloning vectors, sequencing primers and other cloning reagents.

Production of Antibodies

The production and isolation of many different monoclonal antibodies in hybridomas in collaboration with IMP group members, and organizing the antibody production in rabbits with an outside company requires a portion of our working time.

Sequencing and DNA Isolation

With the 16-capillery ABI 3100 Genetic Analyzer, and in particular, with the 48-capillary ABI 3730 DNA Analyzer, we sequenced approximately 38,000 samples in the first 10 months of this year. This demand increased in the last two months and we are expecting a further increase in the future not only due to the increased number of new customers, but also due to more new groups at IMBA and IMP.
We are primarily using the 3730 DNA Analyzer because of its sensitivity and its lower running costs. The average read-length is 700-900 bases for standard DNA samples with both Genetic Analyzers equipped either with 80-cm capillaries of ABI 3100 or 50-cm capillaries of ABI 3730.
A problem that is becoming significant is that DNA sample quality and, even more significantly, the concentration of DNA of samples corresponds to our skills in an inverse relationship and thus, the short "return time" (preparing the sample and getting results) leads additionally to increased trial-and-error and multiple-sample sequencing.
We are still using the same easy and fast clean-up protocol with Sephadex G50 superfine columns on a 96-well microtiter plate format, and we could eliminate, in most cases, the so-called "dye-blobs" in the sequencing reaction samples by further optimizing the Sephadex consistency and the centrifugation conditions.

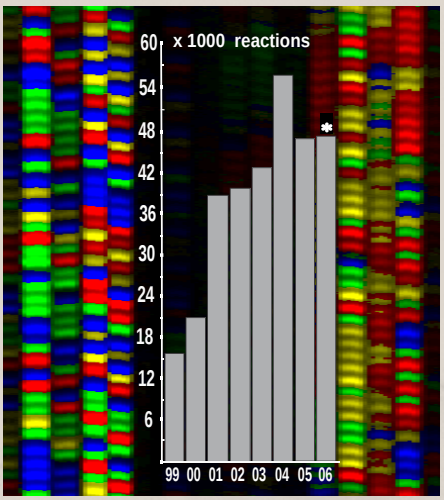

Figure 1: *A sequencing run on an ABI 377 PRISM and number of reactions analyzed on ABI 377 (1999 - 2001), on ABI 3100 (since 2001) and on ABI 3730 (since June 2004) done with dye deoxy terminators (v3.0 since 2001) in the years 1999 to 2006 (scale 0 to 60'000). *calculated from January 2006 to October 2006 data*

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Awards

Ebru Demir

Campus Vienna Biocenter PhD Award 2006 (November 2006)

Barry Dickson

Remedios Caro Almela Prize for Research in Developmental Neurobiology (October 2006)

Stephanie Küng

Peter Kirsten Rabitsch Award (October 2006)
Campus Vienna Biocenter PhD Award 2006 (November 2006)

Corinna Wilken

Campus Vienna Biocenter PhD Award 2006 (November 2006)

Seminar Speakers

JANUARY

11 | 01 | 06 Andreas Hochwagen (MIT, Department of Biology)
19 | 01 | 06 Mauro Giacca (ICGEB Laboratories Trieste, Italy)
27 | 01 | 06 Conly Rieder (Wadsworth Center, Cellular Regulation, Albany, New York)

FEBRUARY

02 | 02 | 06 Henry Roehl (University of Sheffield)
09 | 02 | 06 Silke Sachse (Institute for Biology & Neurobiology, Free University, Berlin)
09 | 02 | 06 Richard Marais (The Institute of Cancer Research, London)
10 | 02 | 06 Yoichi Shinkai (Institute for Virus Research, Kyoto University)
16 | 02 | 06 David Van Vactor (Department of Cell Biology, Harvard Medical School, Boston)
28 | 02 | 06 Wolf-Dietrich Hardt (ETH Zurich, Institute of Microbiology)

MARCH

01 | 03 | 06 Andrea Sinz (University of Leipzig)
02 | 03 | 06 Matthias Wilmanns (EMBL Hamburg)
03 | 03 | 06 Morten Lindow (Bioinformatics Centre, University of Copenhagen)
09 | 03 | 06 Akira Shinohara (Institute for Protein Research, Osaka University)
10 | 03 | 06 Albert Heck (Department of Biomolecular Mass Spectrometry, Utrecht University)
16 | 03 | 06 Andrew McMahon (Harvard University)
21 | 03 | 06 Dave Jackson (Cold Spring Harbor Laboratory)
28 | 03 | 06 Jan van Deursen (Mayo Clinic College of Medicine)
30 | 03 | 06 Nenad Ban (Institute of Molecular Biology and Biophysics, Zürich)

APRIL

06 | 04 | 06 Pitter Huesgen (Department of Physiology and Plant Biochemistry, University of Konstanz)
13 | 04 | 06 Thomas L. Volkert (Whitehead Institute for Biomedical Research, Cambridge, USA)
20 | 04 | 06 Masaru Okabe (Genome Information Research Center, Osaka University)
27 | 04 | 06 Joel L. Sussman (Dept. of Structural Biology, Weizmann Institute of Science)

MAY

05 | 05 | 06 Anuhar Chaturvedi (Department of Hematology, Hannover Medical School)
10 | 05 | 06 Owen Sansom (Beatson Institute for Cancer Research, Glasgow)
11 | 05 | 06 Claude Desplan (New York University, Dept. of Biology)
12 | 05 | 06 James Langridge (Waters Corporation, Manchester, UK)
12 | 05 | 06 Matthias Wilm (EMBL, Heidelberg)
18 | 05 | 06 Juan Valcarcel (ICREA & Ctr de Regulacio Genomica, Barcelona)
19 | 05 | 06 Harold Varmus (Memorial Sloan-Kettering Cancer Center, New York)
24 | 05 | 06 Bill Hahn (Dana Farber Cancer Institute)
29 | 05 | 06 Montserrat Corominas-Guiu (Dept. of Genetics, University of Barcelona)

JUNE

01 | 06 | 06 Helen Blau (Baxter Laboratory in Genetic Pharmacology, Stanford University)
06 | 06 | 06 Andrea Vortkamp (Centre for Medical Biotechnology, University Duisburg-Essen)
06 | 06 | 06 Jean-Pierre David (Rheumazentrum, Berlin)
08 | 06 | 06 Fiona Watt (ICRF, London)
14 | 06 | 06 Jacques Neefjes (Division of Tumor Biology, The Netherlands Cancer Institute, Amsterdam)
16 | 06 | 06 Bernd Fritzsch (Dept of Biomed Sciences, Creighton University, Omaha)
21 | 06 | 06 Frank Uhlmann (Chromosome Segregation Laboratory, Cancer Research UK, London)
28 | 06 | 06 Erik Sahai (Tumour Cell Biology Laboratory, Cancer Research UK)
29 | 06 | 06 James Zheng (Dept. of Neuroscience and Cell Biology, University of Medicine and Dentistry, New Jersey)

JULY

03 | 07 | 06 Catherine Dulac (Howard Hughes Medical Institute, Harvard University)
04 | 07 | 06 Jody Haigh (Department for Molecular Biomedical Research, Ghent University)
06 | 07 | 06 Alexander Tarakhovsky (Laboratory of Lymphocyte Signaling, The Rockefeller University, New York)
13 | 07 | 06 Li Huei Tsai (Department of Brain and Cognitive Sciences, HHMI, Massachusetts)
14 | 07 | 06 Will Wood (Gulbenkian Institute and Institute of Molecular Medicine, Lisbon)
18 | 07 | 06 Reinhold G. Erben (University of Veterinary Medicine, Vienna)
20 | 07 | 06 Ulrich Hartl (Max Planck Institute for Biochemistry, Department of Cellular Biochemistry, Martinsried)

AUGUST

01 | 08 | 06 Goetz Laible (AgResearch, Hamilton, New Zealand)
04 | 08 | 06 Vigo Heissmeyer (GSF-Institute of Molecular Immunology, Munich)
17 | 08 | 06 Norbert Perrimon (Department of Genetics, HHMI/ Harvard Medical School, Boston)
18 | 08 | 06 Michel Pairet (Boehringer Ingelheim Germany)
22 | 08 | 06 Martin Kupiec (Dept. of Molecular Micro & Biotech, Tel Aviv University)

24 | 08 | 06 Paul Klenerman (Institute for Emergent Infections of Humans, University of Oxford)
31 | 08 | 06 Eckhard Mandelkow (Max-Planck-Unit for Structural Molecular Biology, Hamburg)

SEPTEMBER

12 | 09 | 06 David Drubin (University of California, Berkeley)
15 | 09 | 06 Thomas Steitz (Howard Hughes Medical Institute, Yale University)
15 | 09 | 06 Alex Bird (Max-Planck Institute of Molecular Cell Biology and Genetics)
18 | 09 | 06 Michaela Kress (Department of Physiology and Medical Physics, Innsbruck Medical University)
19 | 09 | 06 Thomas Winkler (Fiebiger Center for Molecular Medicine, Erlangen)
19 | 09 | 06 Ed Sturrock (University of Cape Town)
19 | 09 | 06 Rosemary J. Akhurst (Cancer Research Institute, University of California at San Francisco)
21 | 09 | 06 Gerhard Christofori (Institute of Biochemistry and Genetics, University of Basel)

OCTOBER

03 | 10 | 06 Ada Yonath (Weizmann Institute Rehovot, Israel)
11 | 10 | 06 Mark S. Anderson (UCSF Diabetes Center, San Francisco)
12 | 10 | 06 Winship Herr (Center for Integrative Genomics, University of Lausanne)
16 | 10 | 06 Yosef Rafaeli (National Jewish Medical and Research Center, Denver)
18 | 10 | 06 Michael Ehrmann (ZMB Uni, Duisburg-Essen)
25 | 10 | 06 Pamela J. Green (Delaware Biotechnology Institute, Newark)

NOVEMBER

06 | 11 | 06 Min Zhao (University of Aberdeen, Institute of Medical Sciences)
09 | 11 | 06 David Holden (Centre for Molecular Microbiology and Infection, Imperial College, London)
14 | 11 | 06 Witold Filipowicz (Friedrich Miescher Institute for Biomedical Research, Basel)
16 | 11 | 06 Albert Osterhaus (Institute of Virology, Erasmus MC, Rotterdam)
23 | 11 | 06 Bettina Warscheid (Medical Proteome-Center, Ruhr-University Bochum)
23 | 11 | 06 Frank Grosveld (Erasmus University Medical Center, Faculty of Medicine and Health Sciences, Rotterdam)
24 | 11 | 06 Thorsten Hoppe (ZMNH, University of Hamburg)
27 | 11 | 06 Andreas Trumpp (ISREC, Lausanne)
28 | 11 | 06 Katsuhiko Shirahige (Tokyo Institute of Technology)
28 | 11 | 06 Peter Lobie (Liggins Institute, Auckland)

DECEMBER

04 | 12 | 06 Jürgen Ruland (Technical University, Munich)
05 | 12 | 06 Gregory Jefferis (Dept. of Zoology, University of Cambridge)
07 | 12 | 06 Oleg Glebov (MRC Laboratory of Molecular Biology, Cambridge)
14 | 12 | 06 Holger Stark (MPI for Biophysical Chemistry, Goettingen)
14 | 12 | 06 Stefan Kaufmann (Max Planck Institute for Infection Biology, Berlin)

Spotlight on 2006

IMP IMBA Recess

Every year, IMP scientists meet with members of the Scientific Advisory Board to present their work, discuss their research and look for advice and feedback from their peers. Three days in October are devoted to this strictly internal event. It is a time of extensive preparations and heightened activity.
This year's Recess took place from October 4-6 at the premises of IMP and IMBA, taking advantage of IMBA's impressive winter garden for poster exhibitions and coffee breaks. The SAB members were unanimously impressed by the scientific performance and high standards of the research presented.

Symposium "Timing Age"

As in previous years, PhD students of IMP, together with students of the Vienna Biocenter, organized a scientific symposium in fall. The meeting, which took place on November 2 and 3, was devoted to "aging". World class specialists explored the topic from various angles. Their contributions covered such diverse fields as cellular and molecular aging, systems biology, diseases and therapies, as well as the use of model organisms in the study of aging. The last sessions on both days focused on ethical implications of aging research, giving rise to interesting and lively discussions.
The meeting was a big success, with close to 200 participants attending, many of them from abroad. This series will be continued in 2007 with a symposium on "Molecular Psychology".

EMBO YIP Meeting

The EMBO Young Investigator Programme identifies and supports outstanding life scientists in Europe. Each year, EMBO appoints some 20 new „YIPs", as they are called. With 6 present and former YIPs to its credit, the IMP has one of the highest local concentrations of YIPs in Europe. So this year, when the annual YIP meeting was held outside Heidelberg for the first time, it was the IMP's honor to host the meeting.
From May 3-6 the YIPs met in Vienna. For three intense days, they presented their research in a series of short talks, as well as participating in workshops and round-table discussions.

Press Conference "Tomorrow's Targeted Therapies"

On November 8, an international press conference under the motto "Tomorrow's Targeted Therapies" took place at the IMP. Its aim was to communicate Boehringer Ingelheim's research efforts in the field of oncology and present the most promising compounds for future therapies to the media. More than 60 journalists from Europe and Japan participated in the event. The IMP was chosen as location in order to emphasize Boehringer Ingelheim's strong commitment to basic research. The opportunity to visit labs and talk to the scientists was received very well by the representatives of the media.

PhD Student and Postdoc Retreat

Two new initiatives were started in the summer of 2006. The PhD Students of the Vienna Biocenter organized a retreat in Litschau (Lower Austria) and the Postdoctoral Fellows met at the Augustinian Abbey of Brno (Czech Republic). Both events lasted for two days and were highly interactive, with presentations and poster sessions as well as a healthy dose of sports and fun. Invited guest speakers added an extra dimension to the meetings. Both initiatives were received with enthusiasm by the participants and will now become regular annual events.

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Markus GIGLER — *Chef*
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Farzana UDDIN — *Buffet*
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