University of Zurich
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Self-evaluation Report

Institute of Medical Virology (IMV)

Zurich, December 2005

http://www.immv.unizh.ch

Einleitung

- Diese Vorlage ist ein Hilfsmittel zur Erstellung des Selbstevaluationsberichtes und wird gemeinsam mit der Arbeitsgruppe der evaluierten Einheit an die Gegebenheiten der evaluierten Einheit angepasst.
- Der Selbstevaluationsbericht soll dem externen Expertenteam eine optimale Vorbereitung für die Begehung der evaluierten Einheit ermöglichen. Bitte versuchen Sie, jeden Punkt nicht nur als Bestandsaufnahme, sondern selbstkritisch – im Sinne einer Stärken-/Schwächen-Analyse – zu beantworten. Benutzen Sie auch die Möglichkeit, an die Peers gezielte Fragen zu richten.

- Der Berichtszeitraum umfasst die letzten 5 Jahre, also die Zeit von 2000 bis und mit 2004. (Sollten bis zum Abschluss der Evaluation aktuellere hochschulstatistische Zahlen vorliegen, werden diese im Gesamtbericht der Evaluationsstelle ergänzend dargestellt.)

- Der Bericht ist in drei Teile gegliedert:
- Teil A wird in der Regel durch die Leitung der evaluierten Einheit verfasst und beschreibt die Situation der gesamten evaluierten Einheit. Zum Teil sind darin Angaben zusammengefasst, welche in Teil B ausführlich beschrieben werden. Im Teil A nimmt ggf. auch der Mittelbau aus seiner Sicht zur Nachwuchsförderung und zur Arbeitssituation Stellung.
- Teil B enthält Porträts der einzelnen Professuren sowie der Privatdozierenden.
- Teil C umfasst den Anhang zum Bericht. Hier können weitere Statistiken, Listen, Reglemente, Strategiepapiere, Entwicklungspläne, Verordnungen, Pflichtenhefte, kommentierte Vorlesungsverzeichnisse usw. aufgeführt werden.

- Wie detailliert auf die einzelnen Punkte eingegangen wird, hängt sowohl von der Datenlage als auch vom Stellenwert der Themen aus der Sicht der evaluierten Einheit ab. Sollte die Situation der evaluierten Einheit durch die Beantwortung der gestellten Fragen nicht angemessen dargestellt werden können, sind ergänzende Ausführungen erwünscht.
- Bezüglich der Detailgestaltung des Berichts (Layout, Umfang) gibt es keine Vorgaben. Wir bitten Sie jedoch, keine Abkürzungen zu verwenden und ein Inhaltsverzeichnis einzufügen bzw. das vorhandene zu aktualisieren. Die anzupassenden Textstellen (Name der evaluierten Einheit etc.) sollten geändert und die speziell markierten Hinweise vor der Drucklegung entfernt werden.
- Neben dem Expertenteam haben alle Stände der evaluierten Einheit, die Universitätsleitung, der zuständige Dekan und die Evaluationsstelle Einsicht in den gesamten Selbstevaluationsbericht.

Inhaltsverzeichnis

➢ [Hier bitte ein Inhaltsverzeichnis einfügen oder das vorhandene aktualisieren.]

Part A: Information on the Institute of Medical Virology as a Whole

The Institute of Medical Virology (IMV) comprises a Department for Viral Diagnostics and a Department for Research, which is primarily financed by research grants. A central facility for Electron microscopy (EMZ) and the National Centre for Retroviruses (NZR) share the infrastructure of the Institute, the safety laboratories, sterile kitchen, library, administration and the workshop. Together with the Institute of Medical Microbiology an administration is responsible for accounting for the diagnostics of virology and microbiology.

The Diagnostics for Virology is primarily responsible for the University Hospital, Zurich, and surrounding hospitals. The Diagnostics is constantly improved by introducing modern techniques such as the polymerase activity reaction (PAR) for various viruses including pox or newly emerging viruses. Several new tests have been introduced throughout the recent years such as diagnostics of e.g. Noro Viruses. Furthermore, special training for the FMH degree, for PhD and MD students, for doctors and technicians is offered on a regular basis. The income goes to the government, which reimburses some of it to the Institute and recently also to the Dean. The Diagnostics Department passed the qualification as GLP (good laboratory praxis) (Akkreditierung).

Teaching and practical courses are organized for medical students together with the Institute of Medical Microbiology. A series of lectures is offered together with the Institute of Experimental Immunology. The Institute of Medical Virology offers lectures and seminars on virology, including HIV and AIDS, molecular biology of tumor cells, on HIV, Influenza, oncogenes, tumor suppressor genes, signal transduction, cell cycle, intracellular transport, as well as gene therapy and biotechnology with and without viral reactions. The teaching is addressed to students of natural sciences, medical students with interest in molecular medicine, as well as students of the Federal Institute of Technology (ETH). A new program (Studiengang) on microbiology has been established between University and ETH with an enormous technology load. The postgraduate PG-course for MD students took place for many years but the structure will be changed. A special meeting was organized by myself on biotechnology in 1997 in order to open up new perspectives for students and postdocs. This topic appeared of high importance then. The meeting was based on experience obtained during my membership of the German Technology Council of the former German Chancellor Dr. H. Kohl, and the Future Fond of the Mayor of the City of Berlin.

Research emphasis is on signal transduction in normal and tumor cells, especially the Raf kinase, which was discovered in my laboratory. It is a regulator of growth but also of differentiation. As a model the compound eye of Drosophila was analyzed in collaboration with Prof. E. Hafen (Zurich University). The significance of Raf kinase-induced genes was analyzed in collaboration with the Max Planck-Institute, Berlin, where the human genomic project is being persued with robotic screening and automatized processing of the information. The Raf kinase plays also a role in senescence and long-term potentiation (memory). We recently demonstrated an unexpected cross-talk between two signaling pathways, involving two proto-oncogenes, Raf and Akt. The balance between the two decides on the fate of the cell to either undergo proliferation or differentiation. These results were published in two papers in *Science* (1999) and two in JBC (2001, 2002). We are presently asking which mechanisms contribute to the non-proliferative state of the cell by down-regulating the Ras-Raf-MEK-ERK kinase cascade, where AF-6 and Bcr are involved.

Another emphasis is the analysis of protein-protein interaction as follow-up of the yeast-two-hybrid system. This was applied to oncogene products and led to the discovery of a transmembrane receptor with a PDZ domain-containing protein, AF-6 (2004 and 2005). AF-6 or other PDZ-proteins negatively regulate the Raf cascade and Src. We demonstrate its role in wound closure and directionality by time-lapse. For analysis of the interaction a close collaboration with the EMZ using confocal laser microscopy and recently time-lapse has been established. *In vivo* selection using a PCR-shuffle procedure was applied to characterize protein interactions. This was published in a paper in *Nat. Biotech.* (1999). Several protein-

protein interaction partners reveal a complex array of communicating proteins and phosphorylation-dependent regulation.

Signal transduction studies have allowed to identify a novel protein, a propeller-type adaptor, which we showed to be an important intermediate as carrier of kinases in the insulin-dependent glucose metabolism. It is a long searched-for missing link in insulin response.

Furthermore, a DNA vaccine approach, originally initiated during a joint appointment with a US-based biotech Company was further persued. It led to a Swiss phase I/II clinical trial against HIV in 1997. The safety of the method was confirmed. DNA vaccines are being analyzed for Influenza A, Cytomegalo virus, mumps, a bunya virus and SARS in close contact with the Diagnostics Department of the Institute. Immune stimulatory sequences and various routes of application are being tested. For understanding the underlying immune mechanism a number of different knockout mice with defects in their immune system are being analyzed. The excellent animal facilities of the Institute offer a unique chance for a contribution to this competitive field. A DNA coding for a cytokine has been approved for a phase I clinical trial together with the Department of Dermatology, Zurich in 2000, and has been finished in December 2003 using GMP-DNA for human use. I was the Sponsor and Scientific Coordinator. Nine late stage malignant melanoma patients have been treated. The therapy showed some efficacy and the results have been published in Human Gene Therapy (2005). Some patient's follow-up studies are in preparation. Furthermore, preclinical results on combination therapies show a much-improved antitumor efficacy. Several compassionate trials are ongoing. Furthermore, a preclinical study on the treatment of tuberculosis was analyzed in collaboration with D. Lowrie, MCR, London and published in *Nature* (1999).

HIV research is focussed on viral replication. A mimick of a viral structure is under investigation as inhibitor of three enzyme activities. The inhibitor is a designer-made oligodeoxynucleotide against one of the most conserved regions of the HIV genome. Targeting this site induces premature cleavage of the RNA genome, thereby completely inhibiting virus replication. The molecular mechanism is reminiscent to silencing by siRNA and may be designated as siDNA. It leads to HIV suicide (manuscript submitted). Furthermore, the chemokine receptors of HIV have been included into our signal transduction studies. A novel interaction partner of the chemokine receptor has been identified involved in receptor trafficking (2004, 2005).

A tradition of the Institute is antiviral mechanisms mediated by Interferon, which was discovered by the former Director of the Institute, Prof. Dr. J. Lindenmann. Interferon is a continuous major field of interest. In particular we are analyzing the molecular mechanism of the antiviral function of the Interferon-induced Mx protein. Some of these studies include transgenic mice.

Retroviral vectors with inducible promoters serve for gene transfer for research and potential gene therapy.

Research is mainly financed by grant money from several national or international sources.

We are hoping for a productive contribution of our efforts to diagnostics, basic research and to the improvement of medical applications.

Karin Moelling

Zurich, December 2005

1 Important Information at a Glance

- Zum Beispiel:
- Personelle Ressourcen
- Finanzzahlen
- Lehre / Forschung / Dienstleistung
- Nachwuchsstatistik
- Etc.

Personelle Ressourcen

The IMV comprises about 40 coworkers some of which are financed by the Canton mainly for Diagnostics. Administrative positions are also from the Canton and shared with the Institute of Medical Microbiology, National Centre of Retrovirology (NZR) and Electron Microscopy (EMZ). Shared are also the workshop, library, and housekeeping. Furthermore, the IMV supplies the infrastructure (sterile kitchen, autoclave, waste disposal, etc.) also for the NZR. An animal house is maintained and shared with several other University Institutes. The EMZ and NZR are financed independently. Research positions at the IMV, for several postdoctoral fellows PhD or diploma students, are mainly financed through grant money from the Swiss National Science Foundation, Cancer League, KTI, EU, BMBF. This amounts to more than 1 Mio CHF/year (see Table 4). Resources for personnel have recently been organized by a so-called global budget, which is fixed and can only be compensated for by the budget for current supplies.

Finanzzahlen

The annual budget of the IMV amounts to about 2 to 3 Mio Swiss Francs. This is required for maintenance of the diagnostics daily routine, in part for the infrastructure of the IMV and a portion also for the current supplies of the research group. Large equipment required for Diagnostics and research is applied for on an annual basis and approved on the basis of need and the University resources. The IMV Diagnostics Department has received approval for quality control (QC) by the Swiss Authorities (Akkreditierung), which is a very demanding burden for the coworkers and team leaders, not only for the initial approval by also for the permanent maintenance of QC. There has been no increase of personnel for Diagnostics since 1993. The numbers of diagnostic analyses have been more or less constant throughout the last five years and amounts to about 45'000 analyses. A change towards more PCR analyses, which we newly developed for various virus isolates improved the quality of diagnostics in respect to speed and precision, and also led to slightly increased financial income. In the future the budget of 2005 will be the basis for 5.5 % new cantonal tax payments annually for the next three years and additional 1.5, 3.5 and + 5 % will go the Dean in the next three years. Furthermore, most recently a 10 % reduction of all diagnostics and tax points was decided upon by the Government. Thus, in 2008 the budget will be down by 20 % in total. This is a novel development. Surplus in the budget above the predicted financial volume at the end of each year has to be returned to the University Financing Office, and the Dean. Deficits will be subtracted from next year's budgets.

Lehre

A major responsibility for teaching is to instruct diploma students, PhD students, and postdocs how to perform excellent research projects and to become scientifically independent. Furthermore, we try to help PhD students to finish their PhD before the age of 30 years. This was in many cases achieved. The thesis can be submitted by accumulation of scientific publications until recently. This has however been changed recently back to the conventional writing of a thesis. In the year 2004 four PhD theses were terminated as well as several diploma theses. Furthermore, to recruit younger students for collaboration we are participating

in a new lecture program with the ETH. For medical students number and topics of the lectures have been determined in the new Medical Curriculum. This has just been renovated by the Medical Faculty. So far about 300 students participated in the Summer semester in this obligatory lectures including several afternoons' laboratory courses. This compulsory instruction is terminated by a written examination, which is evaluated by the Institut für Medizinische Lehre (IML) in Berne. This program also prescribed how many questions can be asked and furthermore define the kind of questions to be asked (multiple choice). Beginning of Winter 2005 the teaching program for medical students will be completely changed.

In context with the newly founded program for ETH students in combination with the University in microbiology we participate by instructing 28 new lectures and a course program on topics such as molecular virology, viral vectors for research and therapy, oncogenes, molecular approaches to vaccination and viral gene therapy. We are presenting this annually for the third time with about a total of 50 students. Half of them give short oral presentations on recent publications in seminars.

Furthermore, we routinely contributed to a study program designated as postgraduate education (PG-Kurs) for medical students to learn about molecular virology, etc. for which we contribute 16 lectures including discussions and presentations by the participants of recent excellent publications. The organization of the PG-Kurs is presently undergoing changes and will not be maintained as such in the future.

For the recruitment of diploma and PhD courses in natural sciences, which I require for research projects of the IMV, 4 lecture blocks with four hours each and each semester are being presented by K. Moelling at the Free University of Berlin since about 10 years. The majority of diploma and PhD students were recruited in this way to Zurich. About 40 to 50 students mainly trained in biochemistry and recently also about 10 to 12 students from bioinformatics participate. About 10 to 20 students per year come to Zurich for 2 months for laboratory courses. The majority of diploma and PhD students of the IMV are recruited this way. Some of them take their final examinations either in Berlin or at the MN Faculty of Zurich, where I have recently obtained the license for examination of PhD students in Natural Sciences. In Berlin I am an External Faculty Member of the Medical Faculty of the University of Berlin, and was awarded an Honorary Professorship by the Charité Medical Faculty of the Humboldt University in October 2005. We had about 10 Diploma students, 5 PhD students out of more than 100 students from Berlin in Zurich.

Forschung

Several research projects are under investigation at the IMV. One of them is close to medicine and refers to gene medicine approaches for prophylaxis and therapy of viral infections as well as cancer. We analyzed the efficacy of naked DNA as therapy or vaccine in diverse cellular systems and preclinical animal models. We analyzed DNA vaccines against Influenza A viruses, SARS, Hanta viruses, and other viruses. This is based on a project with HIV-DNA in four HIV patients, which I initiated in Zurich with a Biotech Company. Furthermore, we are characterizing a gene medicine therapy of various tumors such as malignant melanoma and colon carcinoma. In another application we also used DNA coding for Alzheimer's amyloid beta precursor protein, APP, protein as a treatment against Alzheimer's disease in mice. In context with two EU Projects we are trying to contribute to a DNA vaccine against breast cancer. Furthermore, we selected out of 20 tested therapeutic genes a cytokine, the Interleukin 12 (IL-12), which was most effective in our preclinical models against cancer and to our surprise also as prevention against establishment of metastases. We analyzed malignant melanoma in mouse models as well as grey horses, which finally led to the approval of a clinical study. I was Sponsor and Scientific Coordinator of this study. Nine patients with malignant melanoma were treated with DNA coding for IL-12 intratumorally at the USZ, a study that was terminated end of 2004 and published 2005. We were able to demonstrate some efficacy in patients, which are encouraging enough to continue this study with larger number of patients. Discussions with Swissmedic have taken place. Furthermore,

compassionate trials on individual patients in Zurich and in Germany have been initiated. At the preclinical level we are trying to improve the efficacy of the DNA therapy by combinations with other compounds. A collaboration on a combination therapy has been initiated with the National Cancer Institute (NCI) in Bethesda, USA.

A DNA vaccine against HIV was performed in collaboration with a biotech company from the USA at the USZ and terminated. The result demonstrated that there were no autoantibodies induced in the patients and no serious side-effects.

A compound to inhibit HIV replication has led to new scientific results and indicated a relationship with the very exciting phenomenon designated as siRNA. We designated it therefore as siDNA. We demonstrate a role of the RNase H of HIV in this siDNA-mediated silencing of HIV. I discovered the retroviral RNase H in 1972. This is under intensive investigation. Furthermore, analyses on intracellular interaction partners of the HIV coreceptor CCR5 have been published and terminated within a PhD project. It will be continued by international collaboration.

Furthermore, we are using recombinant viruses for gene transfer and gene therapy. Among these are recombinant retroviruses, especially HIV-based for non-dividing cells and inducible for expression. The induction system, called tet off, allows induction of genes upon request. Furthermore we are using retroviral vectors for the expression of siRNAs for knockdown of genes for the analysis of gene function. We are furthermore using Semliki Forest virus based RNA vector system by means of replicons.

Another emphasis is based on former analyses of retroviral oncogenes. Among these are the serine/threonine specific protein kinase Raf, which was discovered in my group in 1984. This is an essential intermediate in signal transduction of normal and tumor cells and in many species demonstrating that it is highly conserved and universally expressed. The second viral oncogene under investigation is Akt, coding for another protein kinase. We have demonstrated a cross-talk between Akt and Raf. This was shown in breast cancer cells as well as differentiating muscle cells and led to two publications in Science in 1999. Since then several other differentiation systems have been analyzed for this cross-talk and published in follow-up papers. In our group and internationally, the results were unexpected and showed the significance of these two kinases in growth and differentiation. The results indicates that Raf can be an oncogene or a tumor suppressor gene depending on the cellular background, which is therefore of interest in other investigations. Since then this duality has been shown also for other oncogenes. Throughout the last 20 years our own analyses on the Raf kinase can be summarizes in about 20 different signaling mechanisms. We presently focus on antiproliferative mechanisms, which negatively regulate the Raf-kinase cascade for the maintenance of the quiescent state.

Dienstleistung (Diagnostik)

Our clinical Diagnostics is mainly serving the University Hospital, other hospitals in Zurich and surrounding, private laboratories or individual medical doctors. In the year 2004 we received 17'000 specimens for which we performed 45'000 analyses. The diagnostics performs three kinds of tests: antibody detection, virus detection by means of classical methods such as cell-culturing and molecular methods such as polymerase chain reaction, PCR. Furthermore, the Electron Microscopy Center, EMZ, which is associated with the IMV, performs about 30 analyses per year. We have about 30'000 serological tests and about 15'000 PCR analyses per year, which has increased by a factor of 5 within the last 5 years. Additional PCR assays are newly established.

The diagnostics section has been qualified according to EN 45001 and ISO/IEC 17025. The Audit for renewal of quality control accreditation was performed in February 2005 and the approval extended for 5 more years. The head of diagnostics has recently been appointed as examiner for accreditation of other virological institutes such as St. Gallen.

Furthermore, our QC is extended by participation in ring tests offered by European Quality Control Organizations. (NEQAS for serology and virus isolation, QCMD for PCR analysis). Both organizations have given us record for excellent quality.

Recently we have newly introduced PCR diagnostics for Poxviruses, Cytomegalovirus, Norovirus, SARS-Coronavirus, Influenza A H5N1-PCR. In particular Noroviruses PCR has proven useful in 2004 during epidemics in Zurich and Winterthur. We established our own Noro-diagnostics since 2002 and were the only laboratory in Switzerland, which had these tests available. For some virus diagnostics we are collaborating with special laboratories. These collaborations allowed us to characterize putative SARS patients.

The IMV was designated regional laboratory for bioterrorism in the eastern part of Switzerland in 2004. This requires special training, new assays, same reconstructions and additional equipment. It is financed by the University of Zurich and The Swiss Federal Government. Throughout the recent years the IMV was on alert during the World Economic Forum (WEF) in Davos for putative bioterroristic attacks. We established a self-made Pox Virus PCR assay for this purpose.

Nachwuchsstatistik

The Institute has 1 1/2 PhD positions for Diagnostics and on average 3 to 5 postdocs for research. The number depends on approved grant applications and varies. Two of them are senior scientists with some administrative responsibilities (e.g. biological safety, radioactivity, animal rights). It is part of our University obligation in the Department of Diagnostics to train medical students (2 to 3 per year), and for the requirements of FAMH for Diagnostics (about 2 to 3 per year). This implies an obligatory collaboration and education for about two months. In research we have about 3 to 6 PhD students, and about 2 Diploma students. About 10 semester students per year come for 2 to 3 months. These coworkers are recruited from Switzerland or Zurich, from the University as well as the ETH, through Internet, and through a teaching program maintained with the Free University of Berlin, Department of Biochemistry. Some of these stay on for getting degrees.

2 Brief History, Mission, and Vision of the Institute of Medical Virology

The Institute of Medical Virology emerged from the Institute of Immunology and Virology, which existed for 25 years under the directorship of Prof. Dr. Jean Lindenmann. After his retirement the Institute was divided into two parts: the Institute of Medical Virology and the Institute of Experimental Immunology (1990). The latter is directed by Prof. Dr. Rolf Zinkernagel. In 1993 Prof. Dr. Karin Mölling was appointed Director of the Institute of Medical Virology (IMV) and Full Professor of the University. The IMV is associated with the University Hospital Zurich (USZ) but it belonged formally to the Department of Education of the Canton of Zurich (Kantonale Erziehungsdirektion) while the rest of the USZ belongs mainly to the Department of Health of the Canton of Zurich. The Director of the IMV has the status of a guest in the regular meetings of the conference of the directors of the clinical institutes (Klinikdirektoren-Konferenz). In parallel to the IMV exists the Institute of Medical Microbiology (IMM). These two Institutes are linked locally and structurally by a common administration. The personnel of the administration is formally associated with the IMM. Both Institutes also share a scientific library, a workshop and personnel for maintenance of both buildings, which are interconnected. In addition, based on the history of virology, the IMV harbors the Electron Microscopic Centre (EMZ) and an animal house. These two facilities used to be essential for virus diagnostics. The need of an Electron Microscopic Center (EMZ) and animal house has changed towards increased use for research and less diagnostics. The future of the EMZ will change after retirement of its leader, Dr. Bächi (2005).

Furthermore, the IMV harbors the National Centre for Retrovirology (NZR), which is mainly focussed on HIV-confirming second diagnostics. It profits from the infrastructure of the IMV such as high containment facilities (P3 laboratories) the sterile kitchen with an autoclave linked to the P3 laboratories (double-doors), and service. Furthermore, both institutions, the NZR and the EMZ, share our administration. The administration performs the accounting of the diagnostics.

The IMV and IMM have undergone together the procedure of Quality Control (QC) (Akkreditierung) in the year 2000. A re-evaluation after 5 years has just been terminated in February 2005 for the IMV and the IMM. The reevaluation has led to an excellent result and the QC has been extended for 5 more years.

The IMV has to fulfil three responsibilities: diagnostics for virology, teaching and research.

Viral diagnostics is closely linked to viral infections, which have become a focus again after the emergence of HIV (1984). Before the emergence of HIV it was generally assumed that viruses do not have a high impact on public health. This changed after the HIV epidemics. Furthermore, several emerging viral infections supported the importance of the whole field. These viruses comprise for example Influenza, SARS, Noroviruses, Ebola, Hanta-Viruses. Recently even Pox Virus diagnostics had to be re-established in house because of the potential of Pox-Viruses for bioterrorism. The diagnostic part of the IMV is one of several regional laboratories (Regionallabor) of Switzerland for bioterroristic viruses. For this purpose 9 viruses with potential bioterroristic potential will have to be diagnosed within hours to allow health care measures. This is still in progress in 2005. During the World Economic Forum (WEF) in Davos the IMV has been in charge for potential bioterroristic viral attacks throughout the last three years.

The Diagnostics has about 45’000 analyses per year by means of serological assays, 10'000 to 15'000 analyses are performed for direct proof for viruses and the number of molecular diagnostic assays has been constantly increased from about 1'000 in 1999 to about 20'000 in 2004. It is our goal to assure high quality of diagnostics. Therefore, we are constantly implementing modern approaches and are establishing new PCR (Polymerase Chain Reactions) diagnostics. The latter is of higher sensitivity, specificity and faster, once it is established. For quality control we participate in international ring tests (Ringversuche). In the case of a Norovirus outbreak in a hospital in the Canton Zurich (December 2004), the IMV Diagnostic was the only address in Switzerland, including diagnostic companies, where assays for this virus had been established. Not only the USZ but also private doctors or other hospitals can take advantage of the Diagnostics Department of the IMV.

The second emphasis of the Institute is research. It is the goal to produce qualitative excellent research results and to publish them in high quality journals. These are the basis for grant application for external funding because most of the research team is financed through grant proposals. The money comes mainly from the Swiss Nationalfond as well as organizations, which support medical or clinical research such as the Zurich or Swiss Cancer Leagues. Also support from a German Government Project (Bundesministerium für Bildung und Forschung, BMBF) for the development of applied medical questions including biotechnology “Leitprojekt Molekulare Medizin” was very supportive for our research. This was a multicenter 4 years project together with a Biotech Company. Due to my teaching (Lehrauftrag) at the FU, Berlin, and as external Faculty member I was eligible for this grant. Furthermore, the Institute is involved in international collaborations, such as three EU projects. Furthermore, scientific interaction with clinical colleagues is considered an important responsibility of the Institute of Medical Virology. Thus, in additional to questions concerning basic research we are interested in collaboration with medical groups. We are trying to transfer results from basic research to medical applications for the benefit of patients. In line with this goal we developed an anticancer therapy, which we sponsored and tested as a phase I/II Clinical Trial in cancer patients at the USZ, which evolved from our anti-HIV-vaccine project at the IMV. The preclinical and clinical activities are being performed mainly by young MDs who want to extend their education by learning some molecular biology technologies, and by studying preclinical disease models. German Arzt im Praktikum (AiP), were able to get acceptance from the

Aerztekammer for 9 months as part of their program and usually spent a year at the IMV as an important basis for medically oriented research. This possibility does not longer exist.

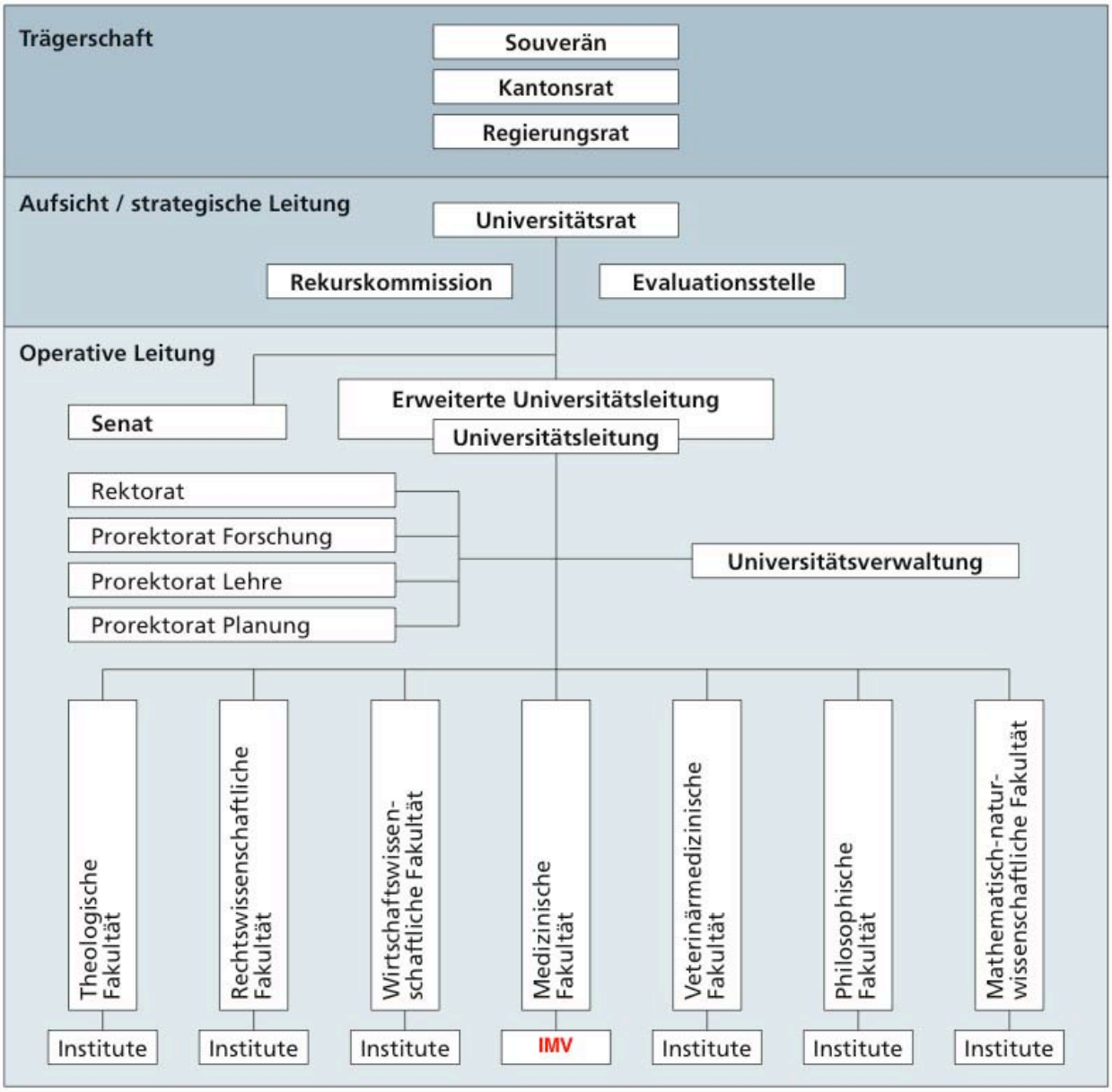
We also consider it important to actively participate in public discussions about public health questions concerning viruses, vaccination or therapies and interact with the press or TV.

Closely connected with diagnostics and research is the education of the coworkers and external students who are constantly trained in molecular biology, molecular virology, recombinant viral vectors for gene transfer for research or gene therapy, molecular approaches for vaccination against viruses and cancer, preclinical animal studies, and applied biotechnology possibilities and finally the design and concept and sponsoring of clinical trials with patients.

3 Structures and Resources of the Institute of Medical Virology

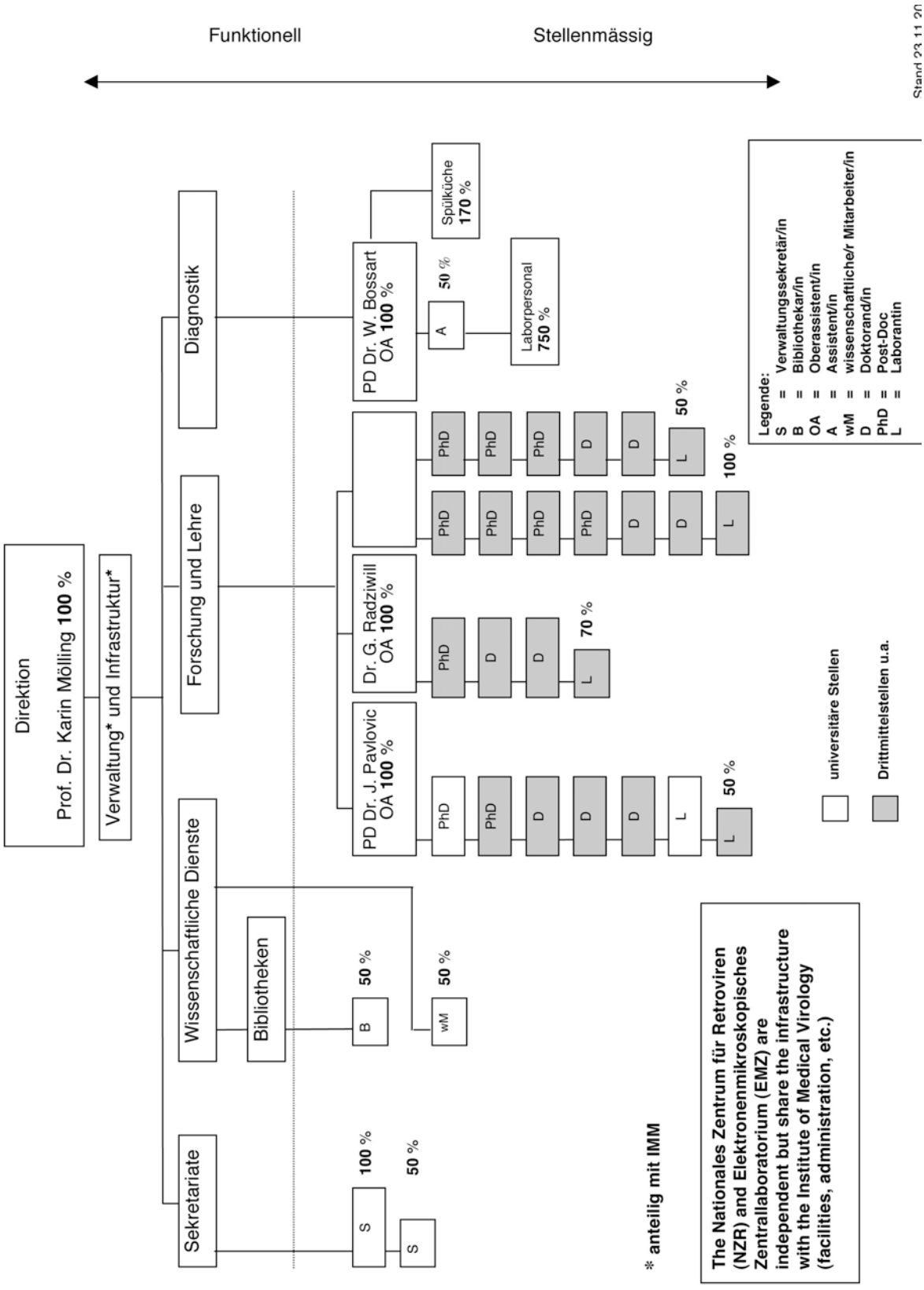
3.1 Structures

3.1.1 Positioning of the unit within larger departments, faculties



3.1.2 Organization chart of the Institute of Medical Virology

mit Angaben von Funktion und Anstellungsgrad



3.2 Human Resources

- Die Personalzahlen werden vom Informationsmanagement und Controlling (IMC) der Universität bereitgestellt. Der Bezug der Angaben vom IMC wird von der Evaluationsstelle organisiert. [Die Tabellengestaltung kann gemäss Datenlage angepasst werden.]

Tabelle 1 : Full-Time Equivalent Positions and Number of Positions, University Funding

Personnel category	Men FTE*	Women FTE*	Total FTE*	Number of Persons	
				w	m
Full Professors	-	1,0	1,0	1	-
Associate Professors					
Full Professors in Second Job (Ordinariat im Nebenamt)					
Associate Professors in Second Job (Extraordinariat im Nebenamt)					
Assistant Professors					
Assistant Professors, Tenure Track					
Graduate Teaching and / or Research Assistants (permanent position)	3,6	0,9	4,5	1	5
Post-Doctoral Assistants	1,2	0,8	2,0	1	2
Assistants					
Other Academic Technical Personnel					
Other Academic Administrative Personnel					
Non-Academic/Technical Personnel	1,0	13,5	14,5	16	1
Non-Academic/Administrative Personnel	-	1,5	1,5	3	-

Tabelle 2 : Full-Time Equivalent Positions and Number of Positions, Third Party Funding by the SNF

Personnel category	Men FTE**	Women FTE**	Total FTE**	Number of Persons	
				w	m
Postdoctoral Assistants	-	1,3	1,3	2	-

As of 31.12.2004
*FTE or **FTE = Full Time Equivalents (sum of all part and full-time positions)

Tabelle 3 : Full-Time Equivalent Positions and Number of Positions, other Third Party Funding

Personnel category	Men FTE**	Women FTE**	Total FTE**	Number of Persons	
				w	m
Graduate Teaching	0,5	-	0,5	-	1
Post Doctoral Assistants	2,8	1,5	4,3	2	3
Non Academic/Technical Personnel	-	0,7	0,7	1	-

As of 31.12.2004
*FTE or **FTE = Full Time Equivalents (sum of all part and full-time positions)

3.3 Material Resources (by P. Blattmann)

3.3.1 Research and Service Infrastructure

Gebäude, Räume, Büroarbeitsplätze, Arbeitsplätze für Studierende

Das Institut ist in einem Aussengebäude der Universität, zentral gelegen im Hochschulquartier von Zürich, an der Gloriastrasse 30, untergebracht. Dieser Bau wurde 1963 als Laboratoriumsgebäude erstellt und ist im “Inventar der kommunalen Schutzgebäude” der Stadt Zürich als schützenswerter Bau aufgeführt.

Insgesamt steht dem Institut eine Hauptnutzfläche von 1300 m^2 zur Verfügung und wird ergänzt durch Räumlichkeiten, die gemeinsam mit anderen Instituten im erweiterten Gebäudekomplex genutzt werden (Bibliothek, Seminar- und mikrobiologischer Kursraum, Probenannahme, Werkstatt, Lager, Verwaltung). Das Institut selbst verfügt neben einer Reihe von modern eingerichteten Standardlabors über zwei Hochsicherheitslaboratorien der Stufe P3, welche Arbeiten mit Infektionserregern höherer Gefahrenstufe und rekombinanten Organismen ermöglichen. Diese verteilen sich über 4 Stockwerke. Ein Stockwerk wurde kürzlich vom Institut für Mikrobiologie übernommen. Die Büroräumlichkeiten (Direktion, Sekretariat, leitende Akademiker) sind mit einem an der Universität üblichen Arbeitsstandard ausgerüstet. Die überwiegende Anzahl der Institutsräume verfügt über Anschlussmöglichkeiten an das Netzwerk der Universität NUZ90. Dadurch ist der Einsatz von modernen Kommunikationseinrichtungen gewährleistet.

Die exakte Einteilung der Räume sowie deren Nutzung sind in den Raumplänen und in den Raumlisten festgehalten (bei Bedarf einsehbar).

Bibliothek (Bestände, Öffnungszeiten etc.)

Es handelt sich um eine reine Institutsbibliothek, welche der Öffentlichkeit auf Anfrage zugänglich ist.

technische Grossgeräte

Das Institut verfügt über eine Vielzahl von Laborgeräten, Apparaten und Automaten. Diese werden – von wenigen Ausnahmen abgesehen – bereichsweise eingesetzt, d.h. sowohl die Diagnostik (akkreditierter Bereich), wie die Forschung verfügen über ihre eigenen Gerätschaften. Für unverzichtbare oder technisch anspruchsvolle Einrichtungen bestehen Wartungsverträge mit dem Hersteller oder den Vertriebsfirmen. Für die laufende Kontrolle der Betriebsbereitschaft sind die Institutsmitarbeiter eigenverantwortlich.

Für die Beschaffung von Neugeräten gilt die Anleitung der Universität Zürich für den Kauf von Investitionsgütern (ab Anschaffungswert von CHF 10’000); ab einem definierten Schwellenwert (z.Zt. CHF 248’950) ist eine Submission durchzuführen.

Diesem Bericht ist in der Anlage ein Verzeichnis der vorhandenen Geräte und Apparate beigefügt, welches Auskunft gibt über Lieferfirma, Kaufpreis und Kaufdatum, Verwendung, Standort, Wartungsvertrag und Gerätehistorie (angefallene Reparaturen).

Ausstattung mit IT

Das Institut betreibt zwei verschiedene Computer-Systeme auf unterschiedlichen Netzwerken.

- Zentralsystem (Labor-EDV)
  Für die Labordiagnostik verwenden wir Hardware von DEC/Compaq und das Softwarepaket MICROS. Der Datentransfer findet auf einem eigenen, unabhängigen Netz statt.
- Arbeitsplatzsystem (Personalcomputer)
  Der Einsatz von PC's oder MacIntosh-Geräten dient ausschliesslich arbeitsplatzbezogenen Bedürfnissen. Die Arbeitsplatzsysteme können auf das Netzwerk der Universität NUZ90 aufgeschaltet werden.

Der Einsatz von EDV ist notwendig für die on-line Verfügbarkeit von Patientendaten des UniversitätsSpitals. Die EDV ist ein unverzichtbares Hilfsmittel, um einerseits die administrativen Aufgaben und andererseits die wissenschaftlichen Arbeiten des Instituts sachgerecht und ohne jeglichen Zeitverlust zu erledigen. Das Zentralsystem gewährt die Einhaltung der gesetzlichen Vorschriften und liefert der Institutsleitung aktuelle Führungsinformationen.

Die Benutzer von lokalen EDV-Einrichtungen handeln grundsätzlich eigenverantwortlich und gewährleisten die regelmässige Sicherung aller relevanten Daten gegenüber dem Arbeitgeber. Im beschränkten Umfang steht den Benützern ein PC-Spezialist zur Verfügung, welcher Unterstützung bei Hard- und Software-Problemen bietet. Er sorgt ausserdem für ein regelmäßiges Update der gängigsten PC-Programme, verwaltet die Benutzerrechte und installiert auf allen Geräten die notwendigen Antiviren-Programme.

Das Zentralsystem wird von Fachspezialisten des Instituts für Medizinische Mikrobiologie betrieben, welches dem Institut eine hohe Verfügbarkeit gewährleisten kann. Es gelten sämtliche Vorgaben für einen ordnungsgemässen Betrieb und Unterhalt eines EDV-Systems.

Am Institut für Medizinische Virologie sind aktuell folgende Hardwarekomponenten installiert:

- 2 Server
- 33 Mac's
- 3 Windows-PC's
- 12 Drucker
- 1 Scanner
- 7 Notebooks
- 3 MICROS-Arbeitsplätze (Labor-EDV)

Es werden folgende Software-Pakete eingesetzt und unterstützt:

MS-Office	Standard-Büro-Anwendungen
Adobe-Photoshop	Bildverarbeitung
Adobe-Acrobat	Dokumentenerstellung
Endnote	Literaturverwaltung
Mac-Vector	Sequenzanalysen
FileMaker	Datenbank
Coreldraw	Zeichnungsprogramm

➢ ...

3.3.2 Expenditures and Income, 2000 – 2004

➢ Die Finanzzahlen werden von der Finanzabteilung der Universität bereitgestellt. Der Bezug der Angaben von der Finanzabteilung wird von der Evaluationsstelle organisiert.

Tabelle 4 : Financial Expenditures and income

	in 1'000 Fr.					
	2000	2001	2002	2003	2004	**in %** 2004
University Funding						
Total Operating Costs	1'494.7	1'749.1	1'702.1	1'515.1	1'622.9	33.39
Personnel Costs*	2'080.4	2'153.8	2'365.3	2'434.5	2'487.6	51.18
Total overhead costs**	508.7	531.9	646.7	649.3	749.5	15.42
Total Expenditure of University Funds	**4'083.8**	**4'434.7**	**4'714.2**	**4'598.9**	**4'860.0**	**100%**
Institute income	-1'860.7	-1'959.9	-1'680.7	-1'992.8	-2'081.7	-42.83
Netto Operating Costs	**2'223.1**	**2'474.9**	**3'033.4**	**2'606.1**	**2'778.3**	**57.17**
Other Funding						
Installation loans / one-time special loans	26.6	17.1	16.4	16.8	15.0	1.78
Third party funds incl. SNF (expenditures annually)***	494.5	608.2	658.6	915.0	825.4	100%
BMBF, "Leitprojekt…." (D)				1999 – 2003:	1.4 Mio DM	

* Sum of: total salary costs excl. professors/excl. social benefits + professors' salaries + social benefits.
** Rent, electricity, water, heating, cleaning, etc. Mieten, Elektrizität, Wasser, Heizung, Reinigung, Entsorgung
*** Total from Table in chapter 6.

Die oben aufgeführten Gesamtkosten pro Jahr werden alljährlich von der Universität als Kredit für laufende Kosten erteilt. Die Zahlen basieren auf den Ausgaben der Vorjahre und Vorhersagen für das kommende Jahr. Diese Betriebskosten werden separat von den Personalkosten gehalten. Letztere sind unveränderbar. Sie werden jedoch jährlich der Teuerung angepasst. Das seit kurzem eingeführte universitäre Globalbudget erlaubt eine Umwandlung der Betriebskosten und Personalkosten im Umfang von 10 %.
Die Verwaltung des IMV erstellt Rechnung für die Virusdiagnostik, die direkt an die Universität abgeführt wird. Sie ist ein Bestandteil unseres Kredits. So wurde z.B. im Jahr 2004/2005 eine unerwartet hohe Diagnostikeinnahme für einen Norovirusausbruch erzielt. Ein Anteil davon in der Höhe von CHF 300'000 wurde im Jahre 2005 dem Institutskredit zugeschlagen. Es kann jedoch auch der umgekehrte Fall eintreten, dass die Diagnostikeinnahmen unterhalb der Erwartungsgrenze liegen, dann wird das Defizit auf das nächste Jahr übertragen. Auch dieses ist bereits eingetreten als Folge von Problemen bei Berufungen im Rahmen der Transplantationsmedizin. Überschuss am Jahresende wird zu 50 % der Universität zurücküberwiesen. Ab 2006 reduzieren sich die Taxpunkte um 10 % aufgrund staatlicher Weisungen. Damit nehmen die Diagnostikeinnahmen automatisch um 10 % ab. Weitere Kürzungen erfolgen durch kantonale Massnamen (3.5 %) sowie neue Auflagen des Dekanats, die für die nächsten drei Jahre gestaffelt 3.5 %, 4.5 % bzw. 5 % Abgabe. Dieses wird voraussichtlich personelle Einsparungen in der Diagnostik erfordern, was dann auch wieder zu verringerten Einnahmen führen wird. Die Regierung hat ab 2006 eine Reduktion der Taxpunkte (Gebührverordnung) auf alle Diagnostikdienstleistungen angeordnet.

3.4 Significant Pending Changes in the Area of Structures and Resources

- Anstehende Rücktritte, Berufungen etc.

Rücktritte

The contract of the director (KM) runs till the end of 2008. This has consequences for the coworkers. Till then it is envisaged to encourage scientific coworkers to apply for a position outside of the Institute. There are only two cantonal positions, which cannot be terminated. These comprise the leader of the diagnostic department and the chief assistant (Oberassistent) who is in charge of safety issues concerning radioactivity, biosafety, biohazards, animal keeping and animal protection responsibilities. It is the goal to make available some free positions for the successor of the Institute to employ some coworkers of his choice. This affects mainly to coworkers on cantonal positions. Also all PhD students should have finished their thesis by this date. Other coworkers of the infrastructure will probably be most welcome because of their experience to any new director.

3.5 Summary of Strengths and Weaknesses in the Area of Structures and Resources (von P. Blattmann)

Strengths	Weaknesses	Measures Needed
Integration im Markt (Diagnostik)	Lange Informations- und Entscheidungswege (Unileitung → Fakultät → (Institut)	
Motiviertes, kompetentes Fachpersonal	Dauerhaft hohe Arbeitsbelastung (zu)viel Administration beim wissenschaftlichen Personal	

Welche weiteren Punkte Stärken/Schwächen gibt es zur Verwaltung (by K. Moelling)

The IMV is well integrated in the University Hospital by participating in case reports, education (Fortbildung), hospital seminar series such as colloquien on Infectious diseases and microbiology, acute problems in immunology and virology and participation in the University Center for Clinical Laboratories (UZL) and a newly founded focus on infectiology.

These interactions are extremely valuable for the interaction with colleagues and were the major reason why the IMV was not translocated to Schwerzenbach. Thus, the central location proved to be very supportive for close contacts with the University Hospital.

The IMV is well integrated into public concerns on infectious diseases and frequently consulted for advice by the press, radio, TV, as well as governmental organizations (BAG). The IMV is frequently consulted for public health measurements concerning acute problems with emerging virus infections (HIV, SARS, Norovirus), and bioterrorism. (e.g. backup for potential bioterroristic attacks during World Economic Summits, Davos).

The diagnostic has to carry a heavy burden due to the quality control requirements and the new requirements for bioterrorism after having been selected as regional laboratory for this area. This requires some reconstructions in the high containment laboratory, constant training of the personnel, new equipment, effort for concentration of the workload of the personnel for instance by automation in order to make available time of the personnel for the new duties.

The virological diagnostics in Zurich is very scattered at different locations. Important areas are not part of the IMV such as hepatitis virology, virological diseases of children and

diagnostics of HIV (which is located at the IMV, but under independent guidance). Furthermore, it is a problem for the diagnostics if appointments of colleagues as professors at the USZ suffer from interruptions for political reasons.

The future of the EMZ is undergoing changes. The present head of the group retired in 2005 and it is unclear whether the EMZ will remain part of the Institute. The EMZ is an important component of the IMV for viral diagnostics, teaching and research, and it is desirable that this will be the case in the future. It will be combined with the Department of Anatomy at the University Irchel. A rotating directorship with the Head of IMV and Anatomy is envisaged in the Structure Plan.

The spacial arrangement of the IMV is a problem. The diagnostic part is located on half a floor, which is very narrow. The whole research team is located on the second floor. Two other floors have recently been attributed to the IMM for the construction of high containment laboratories. They were promised to be reintegrated into Virology later. Further reconstruction for the IMM is expected, which will cause additional restrictions for the IMV. Two laboratories accessible from the backyard only, are constantly being used and essential for the IMV research but are separated too far. Rooms for PCR or microscopy are scattered throughout the IMV building.

I consider it important that the relevance of virology manifests itself by more adequate facilities. The concepts in this respect have changed constantly. The HIV group was promised to move out, the P3 laboratories of the IMV building were transferred to the IMM and have been promised to be returned to the IMV. A new multifunctional building, the Robinson, had been fully planned since 1990. Its realization is unlikely.

Resources

The financial situation, personnel and equipment have been stable throughout the last years. However, budget cuts have been imposed by the Canton as well as the Dean (30 % by 2008) and 10 % on all Diagnostics (Tax-points cut by the Government beginning 2006). This is unrealistic and will reduce the diagnostic capacity – which results then in less benefit for the University or the Dean (50 % of surplus goes to the University).

The excellent leadership by PD Dr. W. Bossart allowed it that the personnel fulfilled the requirements for the QC without increase of positions. Modern automatization allowed us to compensate the workload by technology and automatization.

In the future the virology service function combined with teaching and research is recommended.

Future

It may be worth considering whether a new director of the IMV should be an expert in virology and a medical doctor or trained as natural scientist. The natural sciences were the basis for the development of some new diagnostic tests and novel types of vaccines or therapies. The diagnostics improved and guaranteed quality control for the application of new diagnostic approaches.

4 Management and Administration of the Institute of Medical Virology

4.1 Tasks of Management

4.2 Management Processes

- Art der Gremien / Sitzungen, Häufigkeit der Konferenzen, Mitsprache- und Mitbestimmungsmöglichkeiten der Stände und des administrativen Personals, Institutsordnung

The IMV comprises diagnostics, research, teaching, and administration.

Diagnostics requires a high degree of alertness in respect of acute public health concerns in virology. These are discussed and organized in acute situations by informal meetings with the Head of diagnostics, PD Dr. W. Bossart. For routine maintenance of our duties one meeting per week with PD Dr. W. Bossart takes place routinely. During these meetings we discuss cases, the situation of coworkers, budget, equipment, development of new tests, written announcements, issues concerning space, etc. Dr. W. Bossart as Head of Diagnostics organizes independently all the requirements, which need to be fulfilled for QC and for the „Akkreditierung". These comprise documentation, training of technicians, organization of the sterile kitchen, maintenance of equipment (ELISA machines, light cycler, microscopes, incubators, autoclaves, etc.). W. Bossart summarizes to me the topics of weekly meetings at the USZ, and patient case reports. Minor problems are discussed in spontaneous meetings.

Regular meetings take place with various sub-groups of the scientific coworkers, which are based on research topics or technical expertises. There are about 4 to 6 meetings per week. PhD students are supported predominantly by one scientific advisor, but are also expected to interact with other postdocs for special scientific or technical expertise.

There is an annual meeting, the Institute Kolloquium, which takes place in January for about a week, where every coworker and all students report on their research. They have to answer a questionnaire on questions such as topic, rationale, goal, time frame, self-criticism, criticism to others, actions required. The power-point presentations include a literature survey, data presentation, discussion of problems. Each presentation is followed by an exhaustive discussion by all other coworkers. Everybody has to summarize the bottom-line of this discussion up to the end of the meeting in a written form.

Regular meetings take place with senior coworkers on special topics. Dr. J. Pavlovic is in charge of safety, including biological safety and radioactivity, whereby he is responsible for interaction with the official agencies for applications and reports. Furthermore, he is responsible for instructions of coworkers, e.g. how to use LB2 and LB3 facilities, regular sampling of urine and dosimeters. Dr. Pavlovic is also in charge of the animal house including animal keepers, maintenance of animal house quality and safety.

Dr. G. Radziwill is in charge of maintenance of equipment. He is also informed about all grants and the budget distribution, so that the grant money is distributed according to time frame and topic in the correct way. M. Wiget is in charge of planning of the personnel for grant money, contracts for grant-paid coworkers, guests, patents. Each coworker either or coapplicant is assigned to certain grants for planning and fulfilling the requirements, J. Pavlovic for EU, G. Radziwill for Krebsliga and Schweiz. Nationalfonds. Dr. J. Heinrich was in charge of the organization of the BMBF grant, Leitprojekt Molekulare Medizin. For teaching A. Weiss is in charge of organizing the new Studiengang Microbiology with the ETH including selection of speakers, handouts, selection of papers for oral presentation by the students (around 30 students).

Technicians in diagnostics are under the direction of Dr. W. Bossart. Technicians in research are under the direction of individual postdocs. For each of the above-mentioned topics there is about one meeting per month.

Administration is directed by Mr. P. Blattmann. He is in charge of billing for Diagnostics, of the regulatory affairs of the Cantonal employees (not those from grants), the personnel for

housekeeping, workshop, etc. He makes suggestions for the budget, investments, current supplies. He summarizes the financial situation of the Institute on a monthly bases including a statistics on supply of samples from USZ, other hospitals outside and medical doctors. On the basis of this, a clear evaluation of the present financial situation of the Institute is guaranteed. This is extremely valuable, and rather unique within the University, where many departments of the Hospital do not know their budgets. Meetings with Mr. Blattmann take place once per month. Minor issues are discussed by e-mail.

Interaction with the neighbouring Institute of Microbiology take place mainly by the senior staff and refers to diagnostics questions, sharing of equipment, space, and resources. This is informal and on a very friendly basis. There is no interaction with the leaders of the NZR or EMZ. However, the interaction at the scientific level occurs directly among the scientists and has been friendly and supportive throughout the recent years.

4.3 Personnel Management, Personnel Development

- z.B. Pflichtenhefte für wissenschaftliches und administratives Personal, jährliche Beurteilungsgespräche für die Mitarbeitenden, Zielvereinbarungen, Fort- und Weiterbildung, Förderung der Gleichstellung von Mann und Frau, Berufungskriterien/-prozedere, Gewichtung der didaktischen Qualität bei Berufungen, klimafördernde Massnahmen etc. (eigentliche Nachwuchsförderung in Kap. 7)

Cantonal employees are evaluated on the basis of a long cantonal questionnaire (about 30 questions) in which their duties are listed and the quality of the work evaluated with six grades. Furthermore, questions need to be answered whether any measures or actions are required. Every coworker has to agree to this evaluation.

The head of diagnostics is expected to perform highest quality diagnostics. He is encouraged to take part in national and international meetings, as well as training and education where desired. This comprises about 5 meetings per year. This is strongly encouraged to allow self-evaluation in respect to international standard and to build up contact with colleagues on an informal basis.

Scientific coworkers are expected to perform high quality research at an international standard. For that, national and international meetings are encouraged to be attended. Normally, the minimal requirement to be fulfilled for participation is a poster presentation. This gives every postdoc the chance to be confronted with the international competition by a direct experience. So far there has not been a restraint for anybody to attend a meeting on the expense of the Institute if that was desired. Furthermore, the University and the ETH offer numerous possibilities to participate in high calibre seminar presentations and meetings.

Teaching qualifications are being acquired by each postdoc. All of them are encouraged to participate in teaching, sometimes even students take over teaching under my supervision. Criticism is normally given afterwards from other coworkers or myself.

There are many occasions for informal celebrations, a skiing day is organized, a Christmas party takes place every year, every accepted Nature paper is followed by a Champagne party.

4.4 Administration

- z.B. Administrative Abläufe, Regelung der Zuständigkeiten, Kommunikation, Zusammenarbeit zwischen wissenschaftlichem und administrativem Personal, Belastung des wissenschaftlichen Personals mit administrativen Aufgaben

Administration is organized by Mr. Blattmann and comprises specialists on budget, orders, controlling, etc.

Mr. Blattmann is in charge of billing for Diagnostics, controlling of the budget, organization of the cantonal personnel, fulfilment of regulations for public employees, such as older age

insurances, health insurances, fulfilment of working regulation, interaction with cantonal and university administrations.

Dr. W. Bossart is in charge of maintenance of Diagnostics, interaction with the University Hospital, fulfilment of requirements for “Akkreditierung”, logistics, quality control of all diagnostics processes, communication with doctors upon the diagnostics results, and recording by University computer.

Scientific coworkers are involved in administrative responsibilities to less than 5 % according to their own estimates. Those who are involved in some responsibilities consider this as an important aspect for learning for their future responsibilities. Those who are involved in administration have support by a technician to make up for some loss of time and also are supported financially by diploma and PhD students from the institutional budgets. The person complaining about too much administration is me.

4.5 Cost Controls

On a monthly basis the annual budget is summarized by Mr. Blattmann and his coworkers and given to my attention. The budget planning occurs in collaboration with Mr. Blattmann on the basis of previous budgets and the annual development of the financial situation of the Diagnostics and the Institute and the grant money acquired from outside.

More frequent meetings are performed towards the end of the year with the goal to achieve an even budget. This has been the case throughout all the years.

Budget control of grant money is within the responsibility of myself in cooperation with Mrs. M. Wiget and the respective coworkers. We perform the planning, and the overview, the financial part is supported by Mr. Blattmann and his coworkers, and the Fond Administration of the University.

Tasks of Management; Report by Mr. Blattmann:

Vorbemerkung

Mit Ausnahme der Tätigkeiten des Direktionssekretariates (1,5 Stellen) erfüllt die Verwaltungsabteilung des Instituts für Medizinische Mikrobiologie (IMM) die Administration der evaluierten Einheit. Im Folgenden beschränken wir uns auf das Allerwesentlichste und verweisen auf die Selbstevaluation (in Vorbereitung) der Verwaltung des IMM.

Führungsgrundsätze

Die Mitarbeitenden sollen eigenverantwortlich handeln. Weisungen bleiben auf das Notwendigste beschränkt.

Für die verschiedenen Anliegen müssen die Mitarbeitenden ansprechbar und offen sein. Kreative Lösungsfindungen sind auf allen Stufen des Instituts erwünscht. Sie sollen wirtschaftlich vertretbar sein und einen wesentlichen Nutzen für Lehre, Forschung und Dienstleistung aufzeigen.

Eine hohe Selbstständigkeit und Selbstverantwortung ist ausdrückliches Ziel der Institutsleitung.

Führungsprozesse

Die Verwaltung ist Dienstleister gegenüber der Institutsdirektion und den Mitarbeitenden. Die Institutsdirektion und der Verwaltungsleiter besprechen sich bei Bedarf; wesentlich aber bei finanziellen und personellen Fragestellungen. Entscheidungen werden einvernehmlich gefällt.

Personalprozesse

Jede/r Mitarbeiter/in mit einer kantonalen Anstellung verfügt über einen Stellenbeschrieb (Pflichtenheft), welcher Auskunft gibt über:

- Identifikation (der Stelle)
- wichtigste Arbeitsziele

- organisatorische Eingliederung
- Kompetenzen / Verantwortung
- Aufgaben
- Anforderungsprofil

Neu eintretendes Personal wird am Ende der Probezeit (nach 3 Monaten) und nach dem ersten Dienstjahr vom direkten – auf Verlangen vom nächsthöheren – Vorgesetzten beurteilt. Nach Absolvierung des ersten Dienstjahres erfolgt die Mitarbeiterbeurteilung alle zwei Jahre, allenfalls früher bei Bedarf.

Zielvereinbarungen beschränken sich auf Kaderfunktionen und das akademische Personal.

Für die laufende Betreuung und Weiterbildung jedes einzelnen Mitarbeitenden ist in erster Linie der direkte Vorgesetzte zuständig. Das Institut achtet auf eine ausgewogene Fortbildung der Mitarbeiter/innen, insbesondere was die Förderung der Fachkompetenz betrifft. Die absolvierten Kurse / Schulungen werden im jährlich erstellten Weiterbildungsplan festgehalten.

Innerhalb der Universität hat das Institut den Status eines Experteninstitutes, d.h. sämtliche Personalgeschäfte werden in eigener Kompetenz durchgeführt.

Administration

Der diagnostische Teil des Instituts (sowie die Verwaltung IMM) ist akkreditiert nach der Norm ISO 17025 und untersteht damit geregelten Arbeitsabläufen und –Prozessen.

Die Bereiche Personal, Finanzen, Einkauf, Ausrüstung und Räume verfügen über eine geregelte Struktur, die sich über Jahre bewährt hat oder die von übergeordneten Stellen (Fakultät, Universitätsverwaltung) vorgeschrieben ist.

Die dem Institut zur Verfügung stehenden Kredite und die personellen Ressourcen sind den autonomen Bereichen Direktion, Diagnostik und Forschung zugeordnet und von diesen bewirtschaftet. Der Kostenverlauf wird monatlich anhand der Budgetkontrolle vom Kostenstellenverantwortlichen und Instituts- / Verwaltungsleiter überwacht. Die Verantwortung für die Einhaltung der kantonalen Stellenpläne und der Personalkredite ist dem Verwaltungsleiter zugeordnet.

Vorhandene administrative Ablaufregelungen und Weisungen werden vom Institutspersonal meist – aber nicht immer genügend konsequent – eingehalten. Die Belastung des wissenschaftlichen Personals mit administrativen Aufgaben ist – insbesondere im Bereich der Drittmittelanträge – sehr hoch.

„Cost Controls“

Die Finanzabteilung der Universität stellt dem Fachpersonal des Instituts ausgezeichnete Möglichkeiten (SAP) für ein praktisch zeitnahes Controlling und Reporting zur Verfügung. Zur besseren Überwachung des Materialeinkaufs – und einer möglichst einfachen Präsentation der Budgets für den ungeübten Leser – unterhält das Institut ein einfaches Buchungssystem.

4.6 Summary of Strengths and Weaknesses in Management and Administration (by P. Blattmann)

Strengths	Weaknesses	Measures Needed
Ausgeprägtes Dienst-leistungsbewusstsein bei den Mitarbeitern	Fehlende Kapazität für neue Aufgaben	None
Einfache Arbeits- und Entscheidungsabläufe		
Good interaction with the Administration	Administration and overview of grant money is in charge of	

Good participation of coworkers to fulfil the budget Synergism between the Institutes	Mrs. M. Wiget and Karin Moelling, thus, increase of grant money is increase of administrative burden

5 Teaching

5.1 Framework Conditions

- Stellenwert der Lehre, Verhältnis Lehre / Forschung

Teaching obligations in Medical Virology follow the concept of the curriculum for medical students. The program comprises teaching lessons on Medical Virology, laboratory classes, and a written final exam. The exam is evaluated by the Institut für Medizinische Lehre (IML) in Berne. The IML also makes statistics on the questions and the results, and the success by the students. The curriculum has changed in the year 2005. We have adjusted the curriculum for 2005 according to the new concept, whereby we have been involved in designing this new concept. This course is obligatory for medical students. About 250 to 300 students take part in this program.

Throughout the last three years we have performed teaching for a new teaching and course program within the University (MN Faculty) and ETH on Microbiology. This includes 28 hours of additional teaching mainly in Molecular Virology (in contrast to Medical Virology for the medical students) and laboratory experiments (about one week). This program is embedded into a larger program, to which several university institutions contribute, e.g. Medical Microbiology, Veterinary Virology, Parasitology. About 35 to 40 students participate in this program, the majority coming from the ETH. This will also in the future require oral examinations, which have to be graded. Presently the majority of the students are given a chance to present a high quality up to date publication (e.g. Nature, Science) within 15 minutes. This training is highly accepted by the students.

The postgraduate course (PG-Kurs) involves two days of lecturing from our institute and about 12 students participate in this program, which has been organized throughout the last 8 years by Dr. Zapf, who coordinated the participations of many university institutions throughout Switzerland. The program lasted for 6 to 12 months and was followed by a scientific project in the laboratory of choice of the students. This program, however, will change by the end of 2005 and we will again be involved in the new concept.

A teaching program takes also place at the IMV for PhD students once a week, which is organized by the students. All coworkers of the Institute are encouraged to participate in the above-mentioned teaching programs. This includes also the technicians.

PD Dr. W. Bossart also performs a separate teaching program for the technicians of the Diagnostics Department. This is part of the quality control (QC) of the Diagnostics Department to guarantee the high quality standard of the technicians required.

On an annual basis all coworkers of the IMV are presenting their work to the rest of the institute in a one-week symposium, usually first week in January. Each project is being presented extensively, including state of the art in the literature, results achieved, experiments under planning, time line for the future, examinations or papers envisaged. All coworkers of the Institute are encouraged to contribute their ideas and criticism. Every coworker has to fill in a questionnaire in which he gives his self-evaluation. This intensive meeting is supplemented by some social activities such as evening dinner. The presentations are all performed in PowerPoint and other bases for subsequent discussion in upcoming seminars throughout the following year. They also serve for our printed annual report, which covers almost all ongoing

projects in a modular structure, i.e. a single page per project. This training gives the standard for high quality presentations to all coworkers. Also it is a practice to learn scientific discussions.

We also participate in a seminar together with the Institute of Immunology, which takes place once per week for which we invite one to two speakers.

Similarly, we invite a speaker or give presentation ourselves for the “Colloquium of Infections Diseases” together with the University Hospital.

In order to maintain my APL Professorship and the status of External Faculty Member I teach classes in Berlin which consist of 3 to 4 blocks each semester comprising four hours each (this makes teaching one hour weekly equivalent per semester). I introduced this teaching program to Berlin to attract students to Zurich. The Free University of Berlin is one of 4 Universities with a high quality diploma in Biochemistry and Molecular Biology, recently also in Bioinformatics both of which are numerus clausus programs attracting only the highest quality students. Based on this teaching program we offer a practical laboratory program for Biochemistry students of upper semesters in Zurich for two months. This high standard of the students was very highly accepted by the postdocs of the IMV for small projects (cloning, protein isolations, kinase assays, etc.). About 120 students have participated in this program over the years. We also recruited Diploma and PhD students from Berlin this way. It has always been difficult to recruit Swiss Diploma and PhD students to the IMV. Since about 3 years PhD students and postdocs apply for positions at the IMV on the basis of our Homepage. These come from all over the world. Therefore, the group comprises normally about 6 or more nationalities.

Finally we also trained many MD’s for practical diagnostics within the framework of FAMH for the section Virology. This comprises about 2 months training.

Furthermore, medically oriented applied research was performed very successfully for many years by German “Aerzte im Praktikum” (AIP). This is not known in Switzerland. Young MD’s had to work for 18 months, 9 of which could be performed in the kind of research performed at the IMV. They normally came for 12 months and learnt gene cloning, recombinant DNA technology, molecular virology, gene therapy, and helped develop antiviral and anticancer vaccines. This program was very beneficial for the candidates as well as for the IMV. The program does no longer exist in Germany and was terminated in 2005.

As part of teaching can also be considered the collaboration with members of the medical faculty in translationary research. This involved some common grant applications or scientific collaborations (e.g. oncology, rheumatology, dermatology). The approach to research for MD’s and PhD’s is quite different and involved many scientific discussions.

- Grösse und Zusammensetzung des Lehrkörpers

One full professor, two PD’s (Privatdozent Dr. W. Bossart, Privatdozent Dr. Pavlovic), one OA, and several scientific coworkers who contributed to teaching.

- Stand der Umsetzung des Bologna-Prozesses (gestufte Studiengänge, Anrechnungspunkte)

Here we follow the concept worked out for the curriculum of medical students by a competence team founded by the Medical Faculty. This involves for the future many changes, which we will fulfil.

5.2 Contribution of the Institute of Medical Virology to the Medical Curriculum

- Beschreibung
- It is one of the responsibilities of IMV to teach medical students in medical virology. The teaching concept is part of the new medical curriculum, which has recently been renovated by the medical faculty of the USZ. Within this curriculum we were assigned 30 hours for teaching but 2 additional sections for patients-oriented instructions and teaching as well as laboratory courses. The lectures cover virology in general, structure function, replication of viruses, virus-host interaction as well as individual viruses such as Influenza-, Herpes-, Hepatitis-, Adeno-, Entero- and Retroviruses as well as Virus Diagnostics, viruses involved in pregnancy and children, antiviral compounds, vaccination, tumor viruses and gene therapy.
- Furthermore, the IMV participates in virological colloquium together with the Institute of Medical Immunology, in virological meetings within the institute open to the public (upon announcement). Also the IMV contributes to a log course teaching (about 16 hours for the so-called post-graduate education for MD-PhD program. This latter one may be reorganized soon.
- Liste der Lehrveranstaltungen, an welchen die Angehörigen des IMV beteiligt sind (see enclosures)
- Virology for medical students, part of the medical curriculum.
- Practical course for medical students as part of curriculum
- Lectures course and in the future two case discussions as requested by the new medical curriculum starting in 2005.
- Seminar “Actual Problems of Immunology and Virology”.
- Virological Colloquium.
- Applied Virology, Colloquium Infectiology and Microbiology at USZ.
- MD PhD program (PG-Course) in collaboration with MN Faculty.
- New studying program Microbiology between ETH and University with the contribution in teaching (30 hours) and instructions in a practical course for about 30 students for about 1 week and seminars for students with reports on selective publications about 15.
- Future examinations within this program have to be performed by Karin Moelling.
- Lehrziele
- For the medical students medical as well as molecular virology aspects are the goal, in order to give medical students the basis to understand future development such as modern techniques in diagnostics as well as the basis for gene therapy.
- Education as required for medical students with the emphasis on modern techniques and molecular aspects.
- Lehrmethoden
- For the medical students the methods are mainly based on PowerPoint and overhead projector presentations. Each student gets at the beginning of each lecture a detailed handout covering the whole oral presentation of the lectures. Some of these are the basis for the following examinations. The laboratory course covers up to date diagnostic technologies such as PCR but also immunohistochemistry, immunofluorescence with about one microscope per five students, ELISAs, Western Blots, etc.
- Lectures with overheads or PowerPoint presentations, and handouts for each participant.
- Prüfungen, Qualitätssicherung und Entwicklung in der Lehre
- The number and kind of examination questions are defined from a Swiss program, which are prescribed to us from a central organization from Berne (IML; Institut für Medizinische Lehre, Universität Bern). Also the results of the students who participated in these examinations are

evaluated from Berne and we are informed about the results and potential criticisms based on statistical evaluations (e.g. too simple, or too difficult, ambiguous answers, etc.).

- Furthermore, the students are given questionnaires, also the PG-students give their comments on teaching and topics, which we obtain for consideration.
- The modalities for teaching and examinations are presently undergoing major changes.
- Koordination mit anderen am Medizincurriculum beteiligten Kliniken und Abteilungen
- Our teaching is based on the newly designed medical curriculum.
-

5.3 Contributions of the Institute of Medical Virology to other Curricula

- Beschreibung
- The IMV participates in a common program between University and ETH on microbiology, which was initiated two years ago and attracts 30 to 50 students. We contribute lectures on medical virology, recombinant vectors, gene therapy, molecular mechanisms for cancer, signaling in normal and tumor cells, oncogenes and tumor suppressor genes, molecular vaccination strategies, receptor ligand interaction, proliferation versus differentiation or apoptosis, whereby the molecular level is stressed compared to the teaching program for the medical students. Therefore, the teaching is separate from the medical virology lectures.
- Liste der Lehrveranstaltungen, an welchen die Angehörigen des IMV beteiligt sind
- Signal Transduction in Normal and Tumor Cells and Molecular Virology Lecture Course and Block Laboratory Course in collaboration with ETH.
- Block Lecture Course at Free University Berlin.
- Laboratory Courses for ETH students, medical students, biochemistry students (Berlin) for two to six months.
- Instructions for laboratory research for students as requirements for diploma and PhD.
- Lehrziele
- The students for natural sciences or ETH students are instructed to learn up to date molecular mechanisms in cell biology, virology, gene therapy, etc.
- Lehrmethoden
- For the students of natural sciences molecular and biochemical details are presented. Also the students get booklets carrying the whole presentations of the instructors with all overheads or PowerPoint pictures. These are distributed to the students at the beginning of the lecture course.
- In particular the reading obligations or short seminar reports proved to be of high acceptance for the students. The basis for the seminars are 10 to 15 minutes presentations with subsequent discussions with mainly *Nature* or *Science* papers from recent years. Oral presentations are also chosen to replace oral examinations in particular for bioinformatics students, about 15 per semester, in Berlin.
- Prüfungen
- The medical students have to write examinations at the end of the semester, whereby Virology is part of an examination covering also Microbiology, Parasitology and Immunology. The questions have to follow special designs prescribed by the central examination office for medical students of Switzerland in Berne. Also the number of questions is prescribed. After the examination a statistical evaluation is distributed to us. This evaluation may lead to changes for the future or even for the past examination in case of inadequate questions. Thus, this examination is highly standardized.
- Oral presentations mentioned above are not yet part of examination in Zurich but only in Berlin.
- Qualitätssicherung und Entwicklung in der Lehre

- Quality insurance is included by consideration of modern publications in the field or actual occurrences such as virus outbreaks or public health problems in Switzerland or other parts of the world.
- Teaching can always be improved.
- Koordination mit anderen beteiligten Instituten
- The Virology Lectures are part of the medical program and are coordinated with the other institutions to avoid redundancies and overlaps or to avoid gaps.
- The Virological Colloquium is mainly organized by the Institute of Immunology, the colloquium for Infectiology and Microbiology is organized by various colleagues. The PG-Course will be organized by the MN Faculty in the future. The Virology Lecture and Course for University and ETH students is also part of a whole program on Microbiology as a new course program.
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5.4 Teaching Load of the Institute's Staff

Type of Course	Number of Hours per Week per Semester				
	2000	2001	2002	2003	2004
Prof. Dr. K. Mölling					
- Lecture Course	2-3/week	2-3/week	2-3/week	2-3/week	2-3/week
- Others	3	3	3	3	3
OA Pavlovic					
- Lecture Course	1/week	1/week	1/week	1/week	1/week
- Others	1/week	1/week	1/week	1/week	1/week
OA Bossart					
- Lecture Course	1/week	1/week	1/week	1/week	1/week
- Others	1/week	1/week	1/week	1/week	1/week

- Hier sind nur die Lehrveranstaltungen aufzuführen, welche durch die Professur / den Lehrstuhl ggf. die Forschungsgruppe selbst durchgeführt wurden. Allenfalls im Text Hinweise auf gemeinsam mit Anderen durchgeführte Veranstaltungen. Stundenangaben nur für die Durchführung, ohne Vor- und Nachbereitung

5.5 Summary of Strengths and Weaknesses of Teaching and Academic Program

Strengths	Weaknesses	Measures Needed
Very up to date topics, Methods, health care problems (virus outbreaks, vaccine development, bio-terrorism), up to date papers for seminar presentations, acute virology problems, international standards through participation in international meetings	None	All presentations by PowerPoint are almost finished. Pedagogical aspects

Strengths:

For teaching we offer very modern technologies including gene therapy approaches, recombinant DNA techniques in addition to highly sensitive viral diagnostics. The medical

students perform experiments for determination of CMV infections. They run PCR techniques, perform western blots, and learn how to evaluate virus-infected cells by using immunofluorescence. We have 12 highly qualified fluorescence microscopes for this training. Also the lectures focus on principles to increase fundamental understanding rather than learning numerous facts.

For the ETH and MN Faculty students molecular virology rather than medical virology is the focus and is covered in about 30 hours within the new training program on microbiology. Here we try to increase the understanding for viruses as genetic tools, as organisms, which are highly specialised in interacting with host factors, as the most economical genetic elements, as front-runners of molecular mechanisms. This comprises mechanisms such as antiapoptosis, survival, cancer programs, recombinations, splicing, gene shuffling, counteracting the immune system by escape mutations, or anti-interferon activities, etc.

Also medical students enrolled in the PG program (Post-graduate program) get trained in a similar way for about 16 hours. In all cases students are actively involved by reporting and discussing short high quality papers (Nature, Science) to learn how to extract and explain the most important information or technology progress to their colleagues. This is also the case for the students of Biochmistry of the Free University Berlin. About 15 out of 50 students every semester are students of bioinformatics in Berlin who have to take examinations. These are replaced by discussions of recent high quality publications.

Throughout the last 10 years an informal exchange program with students of Biochemistry from the Free University of Berlin has been initiated by myself and maintained. Lack of laboratory exercise facilities created this program in which students participate in laboratory experiments of postdocs or PhD students at the IMV for two months or longer. This training proves to be extremely efficient and the number of interested students meanwhile exceeds by far the space available at the IMV in Zurich. This may be an interesting model for training of biochemistry or molecular biology to students from Switzerland or any other country.

6 Research at the Institute of Medical Virology

6.1 Research at the Institute of Medical Virology

6.1.1 The Unit's Position on Research; Research Goals of the Unit

- Stellenwert und Verhältnis von Grundlagenforschung / Angewandter Forschung / Auftrags-forschung u.ä.
- Research is one of the two major goals of the Institute besides Diagnostics for Virology. Most of the research concerns basic science and to some extent applied science. The latter one mainly refers to contributions to cancer and virus diagnostics, therapy or vaccinations. There is no research through contract by companies. Also in the recent years there is no more any companies-sponsored support of research for cancer or virology, which used to be ten years ago an important financial source for research.
- Beurteilung der Forschungsbeiträge im nationalen und internationalen Vergleich
- Our research results are normally published in international peer-reviewed journals. The quality of the research is envisaged to reach the highest international standards possible. This is not always the case. Some scientific results need to be written up and published even if they do not fit into the top ranking journals. The quality of our international contributions varies to some extend on the quality of the coworkers and the topics under investigation. The highest possible standards are always the goal.

6.1.2 Overview of Research Activities at the Institute of Medical Virology

- Kurzbeschreibung der Forschungsschwerpunkte der evaluierten Einheit
- Focus on research concentrate on:
- Signaling in normal and tumor cells via oncogenes and proto-oncogenes such as Raf, Src, Bcr.
- Antiproliferative signaling by PDZ proteins such as AF-6, CNK, and their role at cellular junctions in normal and tumor cells and metastasis.
- Silencing of HIV by a new mechanism related to siRNA, based on evolutionary relationship between factors of the RISC machinery and replication mechanism of HIV.
- Gene therapy and vaccination against cancer by DNA including clinical trials with K. Moelling as sponsor and principal investigator and coordinator.
- Antiviral defence mechanism against viruses based on the interferon system.
-
- Beschreibung der wichtigsten Forschungsprojekte der evaluierten Einheit

The Raf kinase in signal transduction of normal and tumor cells

C-Raf is the cellular homologue of a retroviral oncogene, which we identified as serine/threonine specific protein kinase in 1984. It is essential in signal transduction from growth factor stimulated receptor tyrosine kinases to the nucleus. It is constitutively activated in cancer and essential during development and differentiation. By subtractive hybridisation we have determinated a number of Raf-activated genes in cancer cells. A focus has been a Raf-Akt crosstalk, which has been published in two *Science* papers in 1999 and several follow-up papers. The balance of the two kinases decides on the cellular outcome, proliferation or differentiation. Using the yeast-two-hybrid system we identified a number of new protein interaction partners, in particular of Akt and PKC. These two kinases interact with a novel propeller-type protein in response to insulin. A recent emphasis has been put on the role of Raf in non-proliferative situations. For the maintenance of epithelial or polar cells quiescence kinases such as Bcr downregulate the Raf-MEK-ERK pathway. Hereby tight-junction proteins such as AF-6 contribute to the formation of monolayers. Some oncoproteins are under investigation for their down- tuning by PDZ proteins, which has also antiproliferative consequences. Bcr is a tumor-suppressor gene, which we recently showed to downregulate β-catenin in quiescent cells. Another signaling project was performed with the HIV coreceptor CCR5 for which a new protein interaction partner JM4 has been identified. It appears to play a role in receptor transport from the Golgi to the plasma membrane.

Signaling via PDZ domains (former funding by Leitprojekt Molekulare Medizin by BMBF, German government)

The yeast two-hybrid system allowed the identification of PDZ adaptor protein binding to various receptors involved in signaling, neuronal pathfinding, angiogenesis, etc., which was published in *Nature Biotechnology* in 1999. Since then PDZ domains have become a focus of research. The results demonstrate that PDZ domains play a major role in the maintenance of a non-proliferative state of cells. They interact with receptor tyrosine kinases and presently one oncoprotein-PDZ protein interaction is under investigation. The PDZ protein AF-6, called canoe or dorsal closure defect in other organisms, and which contributes to the human genetic defect, cleft lip syndrome, is important at tight-junctions for cell-cell-contact. It negatively regulates the Raf kinase pathway for the maintenance of a non-proliferative state. Knockdown by siRNA results in severe cellular changes and prevent spread of viruses. Also the protein Erbin is a PDZ protein under investigation for a number of interaction partners.

We have recently noticed that several well-known kinases have carboxy-terminal bindings sites for PDZ domains. This phenomenon is under intense investigation to understand what kind of consequence binding of the kinases to PDZ proteins might have. We are analyzing the

possibility that this interaction is a negative regulator of the kinase activities e.g. in non-proliferative cellular situations, e.g. monolayers of cells with tight cell-cell junctions where PDZ proteins are known to play a role. We know already that these kinases are under tight enzymatic and spatial control both of which is lost in C-terminal mutants of the kinases. Several PDZ protein candidates are under investigation.

A collaboration with the Charité and Max-Dellbrück-Center in Berlin focuses on peptide ligands interacting with PDZ proteins and other aspects of structure properties of PDZ domains, which is the expertise of this group.

Negative regulation of signaling

Signaling is not only relevant for proliferation or differentiation but requires also control for the maintenance of a non-proliferative quiescent situation. Some of the factors under investigation as negative regulators of growth are tumor suppressors such as AF-6 or Bcr and other PDZ proteins.

We have identified Bcr as a negative regulator of the Wnt signaling pathway. This pathway plays a role not only in proliferation and differentiation but also in stem cell renewal and it is linked to cancer. A central role plays β-catenin, which is not only a transcription factor activating the c-myc gene but also links cell surface molecules such as cadherins to the cytoskeleton for cell-cell adhesion.

We have identified Bcr as negative regulator of this pathway. Bcr can form a complex with β-catenin and negatively regulate transcription of c-myc. Knockdown of Bcr by siRNA relieves the block and activates expression of c-Myc. The expression of Bcr in the colon carcinoma cell line HCT116, which has a high level of β-catenin, leads to reduced c-Myc expression. The negative effect is exerted by the amino-terminus of Bcr and is independent of its serine-threonine kinase activity. Bcr-Abl phosphorylates the amino-terminus of Bcr in tyrosine, which abrogates the binding of Bcr to β-catenin. The inhibitor of Bcr-Abl tyrosine kinase, STI-571 or Gleevec, a novel drug against CML, reverses this effect. The drug inhibits the tyrosine kinase of Bcr-Abl and indirectly restores the tumor suppressor function of Bcr as therapeutic effect. Our data contribute to the understanding of Bcr as tumor suppressor in the Wnt signaling pathway as well as in CML (Chronic Myelogenous Leukaemia).

Recently, the tumor suppressor protein AF-6, which is involved in junction formation, especially in epithelial cells, has been characterized. AF-6 was originally identified as fusion partner in acute lymphoblastic leukaemia. AF-6 is a multidomain protein. We have characterized previously its PDZ domain and showed that AF-6 interacts with Eph receptors, which are relevant for antiproliferative signaling and pathfinding. We showed that AF-6 is located at neuronal postsynaptic membranes. These results were published in *Nature Biotechnology* 1999 and *JCB* 1999. Another domain is the Ras-binding domain, which we characterized as a negative regulator of the Ras-Raf-MEK-ERK pathway in unstimulated quiescence cell, whereas growth factors stimulation led to inhibition of the AF-6 effect and allowed proliferation (*MCB* 2002). More recently we characterized a novel isoform of AF-6, which we were able to knockdown by siRNA and resubstituted by various AF-6 mutants. The result indicated that AF-6 is responsible for formation of cell contacts, wound healing and scattering. A time-lapse analysis of wild type and knockdown cells gave rise to a very surprising phenotype. The cells lost their ability to search for their neighbours and moved straight ahead, which is reminiscent of properties of metastatic cells (JCB submitted).

Negative signaling is presently under intensive investigation with the oncogene/proto-oncogene Src, the oldest oncogene known. We identified a new negative regulatory domain, which restricts properties of the cellular Src to limited selection of substrates. We have characterized several PDZ-containing proteins as ligands of C-Src, which are presently under investigation.

A new transporter of kinases

We have identified a new propeller-type protein consisting of a seven WD-repeat protein and a FYVE domain, designated as ProF. This protein offers a platform for protein-protein interactions by folding into a propeller whereas the FYVE domain localises this platform to vesicles. The gene is conserved among various animal species and expressed e.g. in brain, fibroblasts and adipocytes. ProF binds to the kinases Akt and protein kinases C ζ/λ. After insulin stimulation the kinases become phosphorylated and show increased binding to ProF. The three binding partners colocalize with vesicles containing the glucose transporter 4 in adipocytes and translocate to the plasma membrane upon stimulation. Overexpression of ProF accelerates this process and upregulates glucose uptake. Knockdown of ProF by siRNA leads to reduced glucose uptake early after onset of adipogenesis. Thus, we describe a new conserved binding partner for kinases involved in intracellular trafficking and insulin-dependent glucose metabolism. The protein may improve our understanding of spatial organisation of signaling cascades and be involved in other secretory pathways (manuscript submitted). A second protein, Vamp2, was identified as another interaction partner by ProF. It is also designated as V-SNARE and is phosphorylated by PKCζ upon insulin stimulation. It may allow docking of vesicular structures to the plasma annealing (manuscript submitted). Both topics are part of a Thesis.

Inhibition of HIV replication

HIV replicates via a Reverse Transcriptase and a second enzyme, RNase H, which was discovered in my laboratory. The mechanism of HIV-replication is under investigation and an inhibitor of both enzyme activities has been designed, which shows high potency against various viral isolates – including primary drug resistant patient isolates. The „inhibitor“ of HIV replication turned out to be a mechanism related to siRNA, which we designated as siDNA. The viral RNase H is related to Ago2 (Argonaute protein, consisting of PAZ and PIWI domain). It induces premature cleavage of the viral RNA. Thus, the RNase H is not inhibited but activated to cleave the viral RNA at a wrong time-point and thereby destroys the viral RNA and HIV replication. This is a novel possibility to inhibit HIV replication and could be designated as induction of “suicide”. This mechanism is under investigation. It may be considered for development of drug design. It reduces the virus titer in mice.

A coreceptor of HIV (e.g. CCR5) and a novel interaction partner JM4 is being characterized for signal transduction and possibly interference of HIV infection.

Influenza

Furthermore, we are trying to analyse an inhibitor against influenza virus replication, a new Neuraminidase inhibitor. We have established an animal model for testing various influenza strains in vivo, which was used for analysis of this inhibitor. This analysis was supported by a German grant from the BMBF in collaboration with a biotech company in Heidelberg. The design of the inhibitor is based on a so-called dynamic combinatorial chemistry in conjunction with the Nobel Prize chemist J.M. Lehn, Strasbourg. The animal model is furthermore the basis for our efforts to participate in a novel EU consortium.

Interferon

The analysis of interferon as antiviral defense in the cell was established by the former head of the Institute, Prof. J. Lindenmann. Some aspects of this principle are further analysed by Dr. J. Pavlovic who tries to prove the existence of a so far unknown additional interferon-mediated pathway (see below).

Naked DNA as vaccine and therapeutic against viruses and cancer

Naked DNA expresses low levels of proteins when introduced into the muscle of animals. There it can raise an immune response, which protects mice against viral infections or tumor cell challenge. In small animals it is effective prophylactically as well as therapeutically. Proteins effective at low doses, such as cytokines or hormones, show biological activity in small animals when their respective DNA is injected. Recent results show anti-angiogenesis and inhibition of metastasis. A phase I/II clinical trial against late stage cancer has been performed with patients at the USZ and was terminated (2004) and published (2005) with K. Moelling as Sponsor and Scientific Coordinator. Other trials against cancer are under preparation, also including the NIH, Bethesda, USA. Preclinical studies are performed to improve the therapy, e.g. by combinations. We have accumulated preclinical data on two types of combinations in preclinical studies, which we would like to test in a pilot study in patients. A so-called preIND meeting with Swissmedic has taken place to define the necessary regulatory aspects involved in such a study. This is particularly important because new regulations require GLP conditions also for preclinical data. We are interested in finding partners for the clinical trials.

In order to develop a drug, it is mandatory to raise funds from VC or pharma. This appears to be difficult.

It is the goal of this project to improve the efficiency of the IL-12 treatment by improving gene expression to reduce dose and cost, by testing combination therapies in preclinical animal models, by determinating a new safety parameter, by establishing animal models for testing other cancer indications, and understanding of molecular mechanisms by using knock-out mice. It is the goal of this project to accumulate more preclinical data for future clinical trials. Supported by Krebsliga Zurich.

We are also involved in two projects with an EU consortium on a vaccine against breast cancer, based on DNA technology.

We are very interested in combinations therapies in order to improve the efficacy of IL-12 DNA for local tumor treatment. The combination therapy consists of IL-12 DNA together with DNA encoding other genes or proteins or peptides or known commercially available drugs. The high success out of many tested compounds was observed obtained in the combination consisting of IL-12 DNA with a homing receptors for lymph nodes, CCL21. This dramatically increased the therapeutic and antitumor treatment as well as prophylactic therapy in preclinical tumor models. CCL21 was used as DNA or protein (manuscript submitted). Because of its dramatic efficacy it is our intention to apply a combination in patients in a clinical trial. GMP material for IL-12 DNA is presently produced and its financing guaranteed. We still need GMP material fro CCL21. Inhibitors of B-Raf or the drug Avastin are presently under investigation in combinations protocols in preclinical mouse models. Our results were analysed by Swiss Medic who encouraged us to proceed in patient clinical trials with a combination therapy in Switzerland. It is our intention and hope that the development of the combination therapy will be taken over by some kind of company collaboration or foundation.

Recently we have also prepared IL-12 secreted human mesenchymal stem cells (MSC) and tested the anti-tumor efficacy in preclinical mouse models. The IL-12 was stably expressed in these adult stem cells by retroviral vector. In mice this therapy was not superior to IL-12 DNA treatment (manuscript submitted).

A prime-boost strategy for immunotherapy of breast and ovarian cancer (EU)

One project, an EU multicenter study for the development of an anti breast cancer vaccine has been initiated in 1999 whereby the coordinator is Dr. Joyce Taylor-Papadimitriou at the Imperial Cancer Research Fund, London. The goal is to combine the know-how of clinical groups and groups which are experts on chemical synthesis of glycopeptides and large-scale cell culture expression of glycoproteins. The contribution of the Institute of Medical Virology resides on the development of a DNA vaccine in combination with glycopeptides or glycoproteins. We will perform preclinical studies by testing different DNA/glycopeptide or

DNA/glycoprotein combinations in a murine tumor challenge model. Based on the mucin tumor-associated antigen, MUC1, a cancer vaccine combining a DNA-based formulation with a glycoprotein, and/or glycopeptide immunogen should be developed. To mimic this, the MUC1 antigen will be presented as a DNA-based formulation in combination with a MUC1-based glycoprotein or glycopeptide, reflecting the specific glycoform expressed in cancer.

Development of an immunotherapy for breast cancer based on dendritic cells by developing and comparing different types of tumor specific immunogens (EU)

A second EU-project "Cancer Immunotherapy" aims at the development of an effective immunotherapy for breast cancer, based on dendritic cell (DC) vaccines using the tumor-associated cell surface glycoprotein MUC-1 as antigen. In a first phase of the project this includes the generation of dendritic cells, the production of the breast cancer related immunogen MUC-1, the development of new and the improvement of existing strategies for enhanced antigen presentation by DC's. The objectives of the Institute of Medical Virology as partner of the EU-network "Cancer Immunotherapy" are (i) To establish a strategy for delivery of MUC1-based immunogens by developing virus like particles or viral constructs coding for or packaging the antigen of interest. (ii) To evaluate the possibility for enhancing antigen uptake by coupling of MUC1 to transfer peptides.

Improved methods for virus detection and diagnosis

Viral diagnostic procedures are constantly improved e.g. by PCR technologies. Thereby sensitivity and specificity, but also speed for the diagnosis are improved. We set up a test for pox viruses for the annual World Economic Forum (WEF) in Davos in the case of bioterrorism.

New PCR tests for viruses comprise CMV, Noro, and Influenza H5N1. Other virus PCR assays are under investigation. A recent Noro outbreak has shown that our assay was very useful, since it was the only one available in Switzerland.

The Institute of Medical Virology (IMV) has become a Regional Laboratory for bioterrorism with viruses, which is a heavy burden. The Diagnostic Unit is accredited since five years and got a renewal in 2005.

6.1.3. Overview of the Research Cooperations

- Collaborations of the unit with other research institutions in Switzerland and abroad

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- Research cooperation with companies and associations
- None

6.2 Third Party Funding 2000 bis 2004

- Die Finanzzahlen werden von der Finanzabteilung der Universität bereitgestellt. Der Bezug der Angaben von der Finanzabteilung wird von der Evaluationsstelle organisiert.

Tabelle 5 : Third Party Fundings

	*in 1000 Fr.**					*in %*
Source of funding	2000	2001	2002	2003	2004	2004
SNF Projects	190.5	146.2	197.8	349.7	100.4	12.2
Other competitive programs (KTI, COST; EU Research Programs; University of Zurich Research Credit; other)	232.2	399.6	321.2	408.8	563.8	68.3
Non-competitive programs (gifts, bequests, donations from foundations, contract research)	71.8	62.4	139.6	156.5	161.2	19.5
Total Third Party Funding	494.5	608.2	658.6	915.0	825.4	100%
BMBF, “Leitprojekt….” (D)				1999 – 2003:	1.4 Mio DM	

* Please calculate the sum of funds for all professorships (Part B and C). List spending per year. Please do not count the same funds twice.

6.3 Summary of Strengths and Weaknesses in Research

Strengths

The ongoing research projects require high quality students trained in biochemistry of molecular biology and also medicine whereby the training and quantitative research is preferred. Medical students who jointed into this scientific project are very often highly motivated but have less training. They normally rapidly adopt from those involved in basic research the necessary technologies and often turn out to be more productive and energetic and eager to learn novel approaches than some of the scientists. The IMV strongly took advantage of the German academic medical requirements the so-called „Arzt im Praktikum". This program was also financially extremely valuable for the IMV but equally for the medical students. They normally come for one year, learn molecular cloning, molecular mechanisms of cancer, cancer therapy including preclinical animal studies in our own animal facilities.

Another very strong advantage for the institute is the animal facilities and the high containment (P3) laboratories for characterization of infectious viruses. Furthermore, the close vicinity to the Electron Microscopy group (EMZ) with its excellent facilities such as confocal microscopy, time-lapse, etc. was of great advantage for research purposes.

Furthermore, it is a strength to give coworkers a chance to participate in international meetings or to travel to other laboratories in order to pick up novel technologies.

Furthermore, my participation in scientific networks in Germany increased the international collaboration. This was based on my teaching responsibilities in Berlin, which made me eligible for German Funds, which would normally not have been given to Switzerland.

The diverse projects covered in the IMV were reported and combined by annual meetings where every coworker was given a chance to present his data, discuss various arguments, evaluate his results, his own output, and get advice on new directions, timeline, future possibilities.

Weekly meetings are performed on the basis of topics whereby several coworkers are involved in more than one topic.

It is one of the important goals at the IMV to lead students to the PhD before the age of 30. This was fulfilled for most students. The University allows to take the PhD by the accumulation of two or three original publications, which are then submitted as thesis. This makes the writing of a thesis obsolete. There is a certain disadvantage involved for the Institute because preliminary interesting but unfinished observations are not recorded and tend to get lost.

Weaknesses

The IMV is a separate building and not part of multifloor research institute. This causes some inbred thinking and lack of exposure to other fields, techniques, approaches, and communication with peers. Seminars at the Irchel are well enough accessible. Teaching there failed for me because of lack of interested students. However, the Hönggerberg is too far away for participation in seminars.

The scientific topics covered at the IMV are too diverse. More scientific cooperations might have helped to increase the output and quality of papers.

The selection of coworkers or students at the ETH or Friedrich Miescher Laboratory in Basel is very high and a better chance for success. I tried to set names of some candidates but this was not allowed. The IMV is not good enough integrated into the scientific surrounding – especially basic research is not the emphases of the Medical Faculty. My efforts for a closer integration with the ETH after initial success was blocked. The MN faculty at the Irchel finally allowed me to defend the thesis of my coworkers in basic research.

Measures needed

Academic coworkers should not only be trained as researchers who will perform research for the rest of their lives. Other exit strategies should be considered. The IMV offers many of these strategies but the acceptance is low. This requires support by the University (for example courses or classes).

Another problem manifested itself in collaboration between the IMV coworkers with medical doctors. This is supported by several programs by the SNF. However, the training, the thinking, the interpretation of data, the time available for research by MD's drastically contrasts to that of basic researches. This was the cause for more than one trouble. A better training for MD's in basic research may be necessary or should be envisaged by other measures. We preferred to train MD's within the IMV.

It is very disappointing that the Medical Faculty of the University of Zurich was so frequently a topic in newspapers on lack of quality, lack of management, lack of cooperativity, and scientific misconduct. This was particularly the case for clinical trials. The IMV was involved in a clinical trial with the Head of the Department of Dermatology, which was based on our research. K. Moelling was the Sponsor and Scientific Coordinator, prepared the Clinical Protocol and organized the GMP-DNA. The trial suffered from some problems with the clinical partners. This unfortunate collaboration led to the refusal of four grant proposals applied for by the IMV, which were conceived to extend the preclinical studies, which were the basis for the clinical trials. This caused a severe financial drawback for this research. The conflicts which arose, were certainly not encouraging for the younger generation to persue similar goals. These complications should be handled differently by the University in the future.

7 Promotion of the Next Generation of Academic/Scientific Talent

- Zum wissenschaftlichen Nachwuchs zählen Doktorierende, Postdoktorierende und Habilitierende (universitätsfinanziert, drittmittelfinanziert, externe)

7.1 General Description of the Next Generation Situation at the Institute of Medical Virology

- Förderkonzept
- Diploma students and PhD students are being trained in basic research with the goal of producing high quality papers. Doctors with an MD degree also tend to join the Institute to learn some biomedical research, molecular biology, virology and tumor therapy. They are also trained in modern technology and basic research. This used to be throughout the last five years highly appreciated by „Arzt im Praktikum (AIP)" candidates and was officially accepted for nine months of this training. We used to have normally about 2 to 3 candidates. PhD students were encouraged to finish their PhD training within 2 to 4 years and take the final examinations below the age of 30 years. This was repeatedly successful. Furthermore, the PhD students are encouraged to publish during their PhD time and write their thesis by accumulation of published papers, papers in press or papers written up for submission for publication. This is of high advantage for the students and speeds up the procedure. Sometimes preliminary data are lost by this procedure, which is of some disadvantages for the Institute. Whoever has results sufficient for a poster or oral presentation on national or international meetings is allowed to participate about one time per year on the expense of the Institute. An obligation is a detailed report after return to the Institute. These meetings also serve the purpose to learn about the international standard by direct interaction with colleagues. Furthermore, the Institute supports the students by a high number of modern

computers, excellent research facilities in terms of current supplies, and also technical equipment. Furthermore, even younger students are given the chance to train semester students coming from both Universities in Zurich or Berlin. They normally come for two months. The Institute hosts about 20 semester-students per year, about a 100 throughout the last five years, including even some financial support for living. This gives the coworkers an excellent experience in training younger coworkers.

- Three senior coworkers are supported by technicians to make up in part for their help for administration of the Institute such as budget, planning, organization of lectures and courses, help in recruiting of new coworkers. These are also encouraged to attend international meetings to present their data and be exposed to criticisms. They are also encouraged to write their own grant proposals. Most post-docs always have a PhD student under their guidance.
- Rekrutierung des Nachwuchses
- By homepage of the Institute, Internet announcements, advertisements in *Nature* (only for postdocs), advertisements on meetings, etc. CV, training and previous publications are the basis for the first screening. A personal discussion with referees indicated by the candidates, take place normally by telephone, and candidates are invited for a presentation of their work. Diploma students can report on a project of their choice for a short presentation. For diagnostics the qualification refers to the previous education and experience depending on the position available.
- Beteiligung des Nachwuchses an Lehre, Forschung und Dienstleistungen
- The younger coworkers all participate in research. Teaching obligations are constantly increasing enormously. Teaching has not been allowed for many years, especially not in the obligatory courses. The coworkers participate with big enthusiasm in the MD-PhD course (16 hours per semester) or the ETH course (28 hours per semester). Younger people are not required to participate in diagnostic assays. This requires highly qualified personnel. The accreditation does not allow younger people to be involved except their help is appreciated for the design of assays or techniques, DNA constructs or markers.
- strukturierte Ausbildungsprogram für Doktorierende, Doktorandenkolloquien, strukturierte Program für Postdoktorierende etc.
- On a weekly basis each project is discussed at least once (normally 2 to 3 hours), closely focused on ongoing research. Some coworkers are involved in more than one project and participate in two or even up to three weekly meetings. PhD students organize their own PhD student program. A highly structured program takes place once per year in the annual institutions meeting. This meeting is obligatory for every coworker. A detailed program and instructions of how to proceed in the oral presentations is the basis. This include PowerPoint presentation on the goal of the project, previous results, discussions of the problems, future directions, planned timeline, envisaged examinations or papers, including quality of the journal, and a self-evaluation, as well as evaluation of the whole project. The whole institute participates in very extensive discussions. The meeting is very intense and lasts for at least 6 days interrupted by some breaks. Food and dinner is normally supplied to increase the interaction. The result of the discussion, which sometimes includes some changes for the project is summarized in written by each coworker for future orientation.
- One time I organized a biotechnology meeting with invited speakers in order to open aspects for future jobs in biotechnology.
-

7.2 (Possible: Report by Non-Professorial Staff on Satisfaction with Their Work Situation)

7.3 Statistics on Next Generation Academics/Scientists

7.3.1 Structure of Next Generation Academics/Scientists

- Zahlen werden von der Evaluationsstelle organisiert.

Tabelle 6 : Make-Up of Next Generation Academics/Scientists * 2000 - 2004

	2000		2001		2002		2003		2004	
	Tot.	female	Tot.	female	Tot.	female	Tot.	female	Tot.	female
Doctoral Candidates (Medical Doctor)	5	4	7	4	6	2	8	3	4	3
Doctoral Candidates (PhD)	8	2	8	2	8	2	9	4	5	3
Diploma Students	7	4	2	2	2	2	1	0	2	1
Post-Docs (Medical Doctors)	0	0	0	0	1	1	1	1	0	0
Post-Docs (PhD)	5	0	7	2	6	2	9	3	8	3
Total	25	10	24	10	23	9	28	11	19	10

* Number of persons

(Definitionen:
Doktorierende = Immatrikulierte Studierende im Wintersemester mit Doktorierendenbestätigung im Hauptfach)

7.3.2 Academic Degrees

- Zahlen werden von der Evaluationsstelle organisiert

Tabelle 7 : Academic Degrees Awarded 2000 – 2004 Total

	2000		2001		2002		2003		2004	
Degree	Total	female	Total	female	Total	female	Total	female	Total	female
Ph.D.	1	1	5	4	1	1	5	0	4	2
Habilitation (internal and external)	0	0	0	0	0	0	0	0	0	0
Rejected Habilita-tions (internal and external)	0	0	0	0	0	0	0	0	0	0

7.4 Track Record of Next Generation Academics/Scientists, 2000 – 2004

- Fields of activity, employment following Ph.D.
- PhD students are normally encouraged to leave after they obtained their degree. Sometimes they are employed for about 6 to 8 months to finish writing up their results. Postdocs leave for example to other institutes, e.g. in Zurich, Berne or other places in Switzerland, Germany, USA. Some other coworkers went on to obtain special training, e.g., two were trained in business and administration, one at a diplomatic academy. One coworker left to join a consulting company, one coworker to join a biotech company. One coworker became a schoolteacher. Several went to join research groups at the Hospital. No coworkers so far went to unemployment.
- Track record of the next generation of university teachers
- One senior postdoc will enter the market as university teacher after his habilitation.

7.5 Summary of Strengths and Weaknesses in Promotion of the Next Generation of Academic/Scientific Talent

Strengths

An important factor for the younger generation to be successful is a good training and a PhD examination before reaching the year of 30, submission of PhD thesis by accumulation of scientific publications (published, in press, or submitted), broad education by seminars and lectures and the possibility for interaction with the MN Faculty of the ETH, and the attendance in international meetings, symposia, and workshops, either for training or for presentation of scientific results. The students and young postdocs are furthermore encouraged to gain experience in the presentation of scientific results in seminars or meetings. They are also asked to write grant applications or supported to apply for their own grants. The interaction with medical students occurs through the so-called PG program which, however, will undergo changes in the near future. A new teaching program has been established in collaboration with the ETH, which is very successful. International contacts are encouraged and maintained even for very young PhD students up to the seniors. An exchange program with Berlin improved the international atmosphere of the Institute and allowed the recruitment of well-trained diploma and PhD students. Furthermore, we support the awareness for other fields of employment for scientists such as Biotech companies, patenting, officers, diplomatic, corporate training, founding, additional training in business and administration, science administration, and journalism.

Furthermore, the interaction with the clinicians is strongly supported because the future job openings may be mainly coming for clinical-related research. We are also trying to be involved in public organizations outside Switzerland such as EU, BMBF, US grants, DFG. It is a tendency of research in the future to get the grant money through big networks. It is rather difficult to get into such networks from Switzerland. The number of publications we have obtained from big networks was surprisingly low, which raises some doubts about this kind of policy.

Another strength for the next generation comes from the fact that the Institute was involved in KTI (KMU) project, which involved company interaction partners, whereby the finances came from the Swiss Government. This training teaches younger coworkers differences in thinking between basic research and industry.

Furthermore, the IMV organizes a Clinical Trial Phase I/II on a gene medicine therapy completely developed in house, which was performed at the University Hospital with nine late stage cancer patients. KM was the sponsor and scientific coordinator of this trial. This gave coworkers the possibility to learn about the implications of a clinical trial (such as Swiss Authority, Ethics Committees, GMP production, clinical protocols, evaluation of case report forms of patients, and patent implications). Also the international regulations like FDA or EMEA were considered and brought to the attention of the younger generation.

The institute even employed a “business developer” as a postdoc with the intention to further develop clinical trials and evaluate the possibility for approval or limited approval of the compound. A Pre-IND meeting showed that this required the foundation of a spin-off company. The performance of clinical trials of the University Institute according to the new regulations (2002) has become almost impossible and should be run by a biotech company. Therefore, for the search for corporate partnerships, start-up funds and search for venture capital have been pursued for almost two years. This could have given coworkers a chance to be involved in the start-up company. However, the Biotech situation is not encouraging.

In summary, the IMV tries to offer the following training possibilities: excellent research, clinical collaboration, writing of clinical protocols, performing and evaluating of clinical results (case report forms), good laboratory practice (GLP), good manufacturing practice (GMP) collaboration with small companies (KMU), Leitprojekt Molekulare Medizin with BMBF Germany, KTI (Kommission für Technologie und Innovation), collaboration with Kommission für Klinische Studien (KKS), a new instrument installed in German University Hospitals, writing and persuing of patents, interactions with European and American Patent Offices (exchange

of information with patent attorneys), (Unitectra) Technology transfer office of the University, new funding conditions for Biotech Companies, writing of business plan, interaction with authorities such as Ethics Committees, EMEA, Swissmedic, Clinical research organization, business developer, venture capitalists. The first meeting on biotechnology at the University of Zurich was organized by the IMV in 1997.

Weaknesses

In spite of this variety, in training most coworkers are still fixed to roll-models, which are out-dated.

The University training does not support innovative approaches for alternative careers. The young generation of coworkers is very reluctant in reflecting the different possibilities and offers. The common thinking is – I want a permanent job at the University -. It is almost impossible as an individual person to counteract such a career planning. This should be an important obligation for any university structure to change this way of thinking.

The University has an Ombudsman institutionalized. Frames for decision processes for claimed coauthorship from former coworkers should be set up. Rules for coauthorships may be helpful. I would e.g. suggest that coauthorship requires active support of a paper until it is published. The paper Fritzius et al. has a number of coworkers, close to numbers of coworkers required for sequencing of the human genome, but Mr. Th. Fritzius performed 80 % of the work! Here changes in publication policy should be considered. Similarly, a postdoc who left seven years ago and was without contact, claimed coauthorship. This we accepted in order not to delay the publication. Yet it is unfair for those who did the work. Decisions take six months and block publications. These should be changed.

Measures needed

The young generation of coworkers need to be trained for a higher awareness of time and money, quality standards, international competition. The university atmosphere is far too protective. The pressure should not come from a single boss but from the general surrounding. Only those who have a passion should go into research. This selection is almost impossible to perform successfully for a professor when he employs a PhD student. Thus, too many PhD students should not even have entered this program.

I could foresee the necessity for workshops in which qualifications, life programs of individual expectations and chances for future development should be openly discussed in order to disillusion people from wrong hopes and expectations.

Recruitment of PhD students should follow the selection procedure of the ETH.

8 Diagnostics (by PD Dr. W. Bossart)

8.1 Diagnostic Service

Spectrum of methods:

The methods used for virus detection comprise conventional as well as molecular methods:

Principle of measurement:	Test method:
Detection of infectious virus	Virus isolation in cell cultures including shell vial cultures followed by virus identification by: - Immunofluorescence - ELISA (antigen-capture, sandwich-type) - Passive agglutination - PCR / RT-PCR - Neutralisation assay
Antigen detection	- Immunofluorescence - ELISA (antigen-capture, sandwich-type) - Passive agglutination
Genome detection	- PCR / RT-PCR followed by gel electrophoresis - PCR / RT-PCR followed by hybridisation and EIA - Real-time PCR / RT-PCR in TaqMan® format
Visualisation of virus particles	Electron microscopy (done by third party in-house)

The methods used for anti-virus antibody detection are the following:

Principle of measurement:	Test method:
Antibody detection	- ELISA - Immunofluorescence - Neutralisation assay (neutralising antibodies against poliovirus 1/2/3) - ISAGA: Agglutination assay for anti-*Toxoplasma gondii* antibodies

Spectrum of analyses:

The full spectrum of methods and analyses, their prices according to Swiss federal regulations ("Analysenliste") and information on the weekday performance are listed on the home page of the Institute in the diagnostics section:

www.imv.unizh.ch => Diagnostic => Analysenübersicht / Tarife / Durchführung

Our spectrum of diagnostic methods and analyses for virus and antibody detection is highly adapted to the specific requirements of the high-tech medicine performed at the University Hospitals. The spectrum of analyses is subjected to constant change due to changing requirements and technical innovations.

As an example of the actual spectrum of routine analyses, the types and numbers of assays for antibody and virus detection performed in 2004 are given in the following two tables:

ANTIBODY DETECTION ASSAYS

Microorganism	Method	2004
Adenoviruses	Complement fixation test*	0
Cytomegalovirus (CMV)	IgG EIA	4'455
	IgM EIA	4'346
Epstein-Barr Virus (EBV)	VCA IgG IF	1'870
	VCA IgM EIA	1'756
	EBNA IgG EIA	1'028
Enteroviruses	Complement fixation test for Enteroviruses*	0
	Complement fixation test for Coxsackie B viruses*	0
Human Herpesvirus 6 (HHV-6)	IgG IF	160
	IgM IF	113
HIV 1+2	EIA (screening HIV 1+2)	242
Herpes Simples Virus	HSV-1&2 IgG EIA	986
	HSV-1 IgG EIA	253
	HSV-2 IgG EIA	265
	IgM IF	970
Influenza Virus A / B	Complement fixation test per virus type*	0
Measles Virus	IgG EIA	765
	IgM EIA	163
Mumps	IgG EIA	717
	IgM EIA	164
Parainfluenza Virus 1/2/3	Complement fixation test per virus type*	0
Parvovirus B19	IgG IF	329
	IgM IF	319
Poliovirus 1/2/3	Neutralisation test (neutralising antibodies)	4
Rubella Virus	IgG EIA	2'034
	IgM EIA (screening)	550
	IgM EIA (confirmatory test)	56
RSV	Complement fixation test*	0
TBE (Tick-borne encephalitis)	IgG EIA	249
Virus	IgM EIA	253
Toxoplasma gondii	IgG EIA	2'839
	IgM EIA (screening)	2'322
	Confirmatory test: IgM EIA, IgM/IgA ISAGA (neonates)	244
	IgA EIA	1'796
	IgG / IgM / IgA combined	1'353
	IgG avidity	82
VZV	IgG EIA	1'100
	IgM EIA	562
Total number of assays performed		**30'861**

** Complement fixation analyses performed by an external laboratory (Institute of Medical Microbiology, University of Basel)*

VIRUS DETECTION ASSAYS

Virus	Method	2004
Adenoviruses	Isolation in cell culture (incl. shell vials)	103
	Immunofluorescence assay	90
	Latex agglutination assay	40
	Antigen capture-EIA	96
	Polymerase chain reaction	120
Cytomegalovirus (CMV)	Isolation in cell culture (incl. shell vials)	519
	Immunofluorescence assay	523
	CMVpp65 antigen detection assay	2'739
	Polymerase chain reaction (qual. & quant.)	2'110
Epstein-Barr Virus (EBV)	Polymerase chain reaction	212
Enteroviruses	Isolation in cell culture (incl. shell vials)	276
	Immunofluorescence assay	266
	RT-Polymerase chain reaction	11
Human Herpes Virus 6 A/B	Polymerase chain reaction	28
Herpes Simplex Virus 1/2 (HSV-1/2)	Isolation in cell culture	342
	Immunofluorescence assay	323
	Polymerase chain reaction HSV-1/2	711
Influenza Virus A/B	Isolation in cell culture (incl. shell vials)	3
	Immunofluorescence assay	2
	Antigen capture-EIA	128
	RT-Polymerase chain reaction Influenza A/B	50
Measles Virus	Isolation in cell culture (incl. shell vials)	3
	Immunofluorescence assay	3
Mumps Virus	Isolation in cell culture (incl. shell vials)	2
	Immunofluorescence assay	2
Norovirus (Norwalk-like)	RT-Polymerase chain reaction	93
Parainfluenza Virus 1/2/3/4	Isolation in cell culture (incl. shell vials)	3
	Immunofluorescence assay	0
	Antigen capture-EIA	192
Parvovirus B19	Polymerase chain reaction	38
Polyomaviruses: BK / JC	Polymerase chain reaction	53
Rotaviren	Latex agglutination assay	36
Rubella Virus	Isolation in cell culture (incl. shell vials)	5
	Immunofluorescence assay	5
Respiratory Syncytial Virus (RSV)	Isolation in cell culture (incl. shell vials)	6
	Immunofluorescence assay	6
	Antigen capture-EIA	64
	Polymerase chain reaction RSV-A/B	29
Varicella Zoster Virus (VZV)	Isolation in cell culture (incl. shell vials)	68
	Immunofluorescence assay	131
	Antigen capture-EIA	401

Varicella Zoster Virus (VZV)	Polymerase chain reaction	245
Unspecified	Isolation in cell culture (incl. shell vials)	2'348
	Negative IF for respiratory viruses*	696
	Electron microscopy (performed by the EMZ)	8
Total number of assays performed		**11'483**

** Negative shell vial culture and immunofluorescence for Adeno-, Influenza A/B, Parainfluenza 1/2/3 & RSV*

The change in technologies is best demonstrated by the tremendous increase in demand for PCR analyses.

Method	**2000**	**2001**	**2002**	**2003**	**2004**
Number of PCR/RT-PCR analyses performed	971	1'480	1'721	2'942	3'701
Number of PCR protocols available	10 (+2)	10 (+3)	12 (+3)	14 (+3)	16 (+1)

(Numbers in brackets: protocols not yet validated by panels of clinical samples)

Besides the spectrum of routine analyses, a limited but increasing number of assays for detection of exotic or emerging viruses and potential agents of biological warfare or terrorism is available.

Performance of analyses

Analyses are performed on regular weekdays from Monday to Friday and, at a limited level, on Saturday morning. Emergency analyses needed for transplantation are performed overnight, during weekends and celebration days by the Institute of Clinical Chemistry of the University Hospital under supervision of the specialists of the Institute of Medical Virology.

Diagnostic analyses are performed only by trained technicians in positions paid for by the Canton of Zurich. A professional level of performance is guaranteed by qualified education, long-lasting working experience in the field and continuous education on the job.

Prices

In Switzerland the prices for diagnostic microbiological analyses are subjected to legal regulations at the federal and cantonal level. The specific regulations applying for the types of analyses we do are:

- the „List of Analyses" of the Swiss Federal Public Health Office
- the „Kantonale Gebührenordnung" of the Canton of Zurich

Analyses not covered by these two lists (special or new analyses) can not be billed to the customers and will not be reimbursed by the Health Insurance Companies. According to cantonal regulations, analyses performed by us are billed to the hospitals/ physicians in the majority of cases and not to the patients directly.

Since we function as a reference laboratory in the northeastern part of Switzerland, we also have to perform rarely demanded analyses or analyses with a low or now financial coverage.

Reimbursement of the costs by the health insurance companies is by federal laws restricted to institutions fulfilling a whole range of requirements, all of which are greatly fulfilled by the diagnostic branch of the institute (see § 8.3).

Customers

Doing virus diagnostics in the periphery of the Swiss health system basically means doing serology, which is offered by private practitioners and performed by private diagnostic laboratories. If there is more, patients are transferred to the district or cantonal hospital or the next University hospital. By far the biggest customers for us is the University Hospital of Zurich followed by several other large hospitals of the city and the north-eastern region of Switzerland. A limited number of analyses are done on request of private diagnostic laboratories as part of our function as reference laboratory. The private practitioners send virtually no samples for analyses.

Distribution pattern of customers sending samples:

Type of institutions / customers sending samples:	% of samples
University Hospital of Zurich	75 %
Other big hospitals of Zurich (city and country side) - Children's Hospital of the University - City Hospital Triemli - Cantonal Hospital Winterthur - District Hospitals	15 - 20 %
Private diagnostic laboratories	5 - 7 %
Private practitioners	≤ 3 %

This distribution pattern of the different customers sending samples for analyses stayed fairly constant over the last five years and justifies the strategic focusing of the bulk of our resources on the vital requirements of the University Hospital of Zurich.

Introduction of new analyses 2000 - 2004

In the time interval 2000-2004 test development was clearly dominated by the new technology of real-time PCR. An appropriate PCR system (ABI 5700 PCR system from Applied Biosystems) in TaqMan® format and nucleic acid extraction system (MagNa Pure® system from Roche) were acquired in 2001 and 2002, respectively. Existing nested PCR protocols were replaced by real-time PCR protocols in TaqMan® format and new PCR protocols were evaluated. The majority of these protocols were adapted from literature. For a few viruses (VZV, Adenoviruses, partially Enteroviruses) target sequences were derived from on-line sequence data and the protocols validated in-house.

Besides the molecular diagnostics, several new Elisa kits had to be evaluated for different reasons, most often for product replacement. A new Elisa test for differentiation between HSV-1 IgG and HSV-2 IgG antibodies was evaluated.

In 2000 – 2004 the following analyses have been evaluated, validated and moved to the diagnostic routine as part of the spectrum of accredited analyses:

Year	Virus	Analysis
2000	Respiratory Syncytial Virus A/B	Nested RT-PCR, adapted from literature, validated in the frame of a medical thesis
	Parvovirus B19	Nested PCR, adapted from literature.
	Several labile viruses or viruses hard to grow in cell culture	Target sequences for PCR cloned into bacteria.
	Mumps IgM Elisa	Change of product / manufacturer; proof of equivalence.
2001	HSV-1, HSV-2, CMV, EBV, VZV	Qualitative PCR in TaqMan® format, adapted from

		literature. VZV sequences derived from on-line sequence data.
	Enteroviruses	In-house qualitative RT-PCR in TaqMan® format. Target sequence adapted from literature, modified.
	HSV-1/2	HSV-1 IgG and HSV-2 IgG Elisa (focus).
2002	Adenoviruses	In-house qualitative RT-PCR in TaqMan® format. Target sequences derived from on-line sequence data.
	Parvovirus B19	Qualitative PCR in TaqMan® format, adapted from literature.
2003	Small pox – Orthopox viruses	Orthopox consensus PCR (single round PCR followed by agarose gel electrophoresis), differentiation by restriction enzyme cleavage.
	SARS	RT-PCR (single round PCR), later qualitative RT-PCR in TaqMan® format (synthetic RNA suitable as positive control from Ch. Drosten, Bernhard-Nocht-Institute in Hamburg.
	CMV	Quantitative PCR in TaqMan® format from blood, adapted from literature, validated in-house.
2004	Influenza Virus A/B	Qualitative RT-PCR in TaqMan® format.
	Respiratory Syncytial Virus A/B	Qualitative RT-PCR in TaqMan® format.
	Norovirus	Qualitative RT-PCR in TaqMan® format.
	Epstein Barr Virus (EBV), Varicella Zoster Virus (VZV)	Quantitative PCR in TaqMan® format from blood. Quantification by the specimens from the QCMD quality control panels.

8.2 Statistics

8.2.1 Number of clinical samples in 2000 - 2004

The numbers of specimen we get from our customers are limited by their capacity of beds, rooms and staff, which explains the more ore less stable specimen numbers over the years.

The numbers of clinical samples sent for analyses in 2000-2004 were the following:

Type of assay	**Customer**	**2000**	**2001**	**2002**	**2003**	**2004**
Virus detection assays	*Univ. Hospital ZH*	*3'598*	*3'901*	*4'251*	*4'477*	*4'571*
	Other Hospitals	*1'672*	*1'759*	*1'832*	*2'181*	*2'006*
	Private laboratories	*220*	*288*	*277*	*230*	*351*
	Private practitioners	*79*	*39*	*35*	*60*	*54*
	Total	5'569	5'987	6'395	6'948	6'982
Antibody detection assays	*Univ. Hospital ZH*	*8'412*	*8'501*	*8'160*	*8'245*	*8'344*
	Other Hospitals	*1'188*	*1'221*	*1'087*	*1'244*	*1'602*
	Private laboratories	*648*	*618*	*630*	*553*	*378*
	Private practitioners	*53*	*47*	*30*	*17*	*22*
	Total	10'301	10'387	9'907	10'059	10'346
Total	*Univ. Hospital ZH*	*12'010*	*12'402*	*12'411*	*12'722*	*12'915*
	Other Hospitals	*2'860*	*2'980*	*2'919*	*3'425*	*3'608*

	Private laboratories	*868*	*906*	*907*	*783*	*729*
	Private practitioners	*132*	*86*	*65*	*77*	*76*
Total		**15'870**	**16'374**	**16'302**	**17'007**	**17'328**

Numbers of analyses performed in 2000 - 2004

The numbers of analyses performed in 2000 - 2004 were the following:

Type of assay	**2000**	**2001**	**2002**	**2003**	**2004**
Virus detection assays	8'575	9'317	9'747	11'463	11'504
Antibody detection assays	32'501	32'227	30'861	32'122	32'355
Total	**41'076**	**41'544**	**40'608**	**43'585**	**43'859**

Value of analyses performed in 2000 - 2004

The values of the analyses performed in 2000 – 2004 are the following:

Type of assay	**2000**	**2001**	**2002**	**2003**	**2004**
Virus detection assays	618'045	713'600	785'265	962'380	1'117'655
Antibody detection assays	1'240'902	1'246'296	1'194'701	1'236'564	1'243'499
Total	**1'858'947**	**1'959'896**	**1'979'966**	**2'198'944**	**2'361'154**

8.3 Quality assurance / Accreditation

Our diagnostic activities and services are accredited according to **ISO / IEC 17025** which includes compliance with the requirements of **SN EN ISO 9001**. Accreditation was achieved in 2000. In February 2005 we successfully passed the audits for the second 5-year round of accreditation.

At the same time the institute and its diagnostic activities and services are registered and accepted by the Swiss Federal Office of Health which is a legal pre-requirement for reimbursement of costs for our analyses by the health insurance companies.

External quality controls

The reliability of our results is crucial to physicians and patients. To monitor the performance of the test materials and the reliability of the results we routinely participate in proficiency programs of international quality control organisations (NEQAS= *UK National External Quality Assessment Scheme for Microbiology*, QCMD= *Quality Control for Molecular Diagnostics*). The number of quality control schemes in which we participate is steadily increasing according to their availability by these organisations.

In 2004 we passed the following external quality controls:

	Virus / Type of analysis	**Numbers of distributions / samples per year**	**Quality control organisation:**
Serology	HIV 1+2 Antibody Screening	3 / 18	NEQAS
	Immunity Screening	2 / 12	NEQAS
	Rubella IgG Serology	2 / 12	NEQAS

	Exanthema Screen	1 / 3	NEQAS
	Hepatitis Screen	2 / 6	NEQAS
	Toxoplasma IgG Serology	3 / 18	NEQAS
	Toxoplasma IgM Serology	2 / 8	NEQAS
Virology	Virus Identification	2 / 8	NEQAS
PCR	Cytomegalovirus	1 / 12	QCMD
	Epstein-Barr Virus	1 / 8	QCMD
	Enterovirus	1 / 12	QCMD
	Herpes simplex Virus 1/2	1 / 12	QCMD
	Varicella Zoster Virus	1 / 12	QCMD

QCMD: Until today we analysed all quality control panels with the exception of the Enterovirus panels correctly with only minor discrepancies.

NEQAS: Routinely we reach very high numbers of points relative to the maximum points accredited. Discrepant results until now were test-immanent and, thus, did not require corrective measurements. Our overall performance for combined for serology and virology samples usually is rated by NEQAS above the mean of all participants.

Audits

Internal audits are performed by qualified auditors at a regular basis to monitor the diagnostic performance and check the quality assessment system for inconsistencies and weaknesses.

In the frame of accreditation external audits are performed since 2000 at yearly intervals by representatives of the Swiss Accreditation Service and a reputed virologist (PD Dr. W. Wunderli from Geneva).

Professional education

Academic persons heading the diagnostic activities have a qualified background in either biology or medicine, have passed special education in medical microbiology and have the specialist titles (FAMF) required by law including the supplementing title needed for molecular diagnostics. Continuous professional education is required by the FAMH organisation and is guaranteed by visit of and participation in education event of the university hospital, national societies and companies, and by congress visits at the national and international levels at least once a year. Further, the academic persons heading the diagnostic activities are involved in the teaching of the students and partially responsible for the practical courses for medical students.

All technicians have a qualified professional education as medical technicians and long-lasting experience in medical microbiological diagnostics. Continuous professional education is guaranteed by qualified learning on the job, in-house teaching and visits of external education events organized by the university, societies and companies.

8.4 Usability of the Diagnostics for Research, Teaching, and Promotion of the Next Generation of Academics/Scientists

Usability of the Diagnostics for Research

There is no obvious profit from diagnostics for research. A benefit may come from learning from the quality assurance system implemented in the diagnostic section.

Usability of the Diagnostics for Teaching

Aspects of clinical virology and diagnostics are inherent parts of the lectures and courses of the institute.

Virus diagnostics is the dominant theme of the practical courses for medical students and highly depends on clinical material accumulated in the clinical laboratories.

Active participation in the clinical case presentations and professional education events of the University Hospital require professional involvement in diagnostic activities.

Usability of the Diagnostics for Promotion of the Next Generation of Academics/ Scientists

The institute offers possibilities for medical theses and professional training in virus diagnostics for FAMH candidates.

The following medical students did their medical theses in the field of virus diagnostics:

- Cand.med.: Nicole Chantal Zurflüh:
  Title of thesis: Nachweis des JC-Virus im Liquor cerebrospinalis mittels der Polymerase-Kettenreaktion zur Diagnose der progressiven multifokalen Leukoencephalopathie.
  Accepted: January 2001
- Cand.med.: Susanne Anna Abels:
  Title of thesis: Reliable Detection of Respiratory Syncytial Virus infection in children for adequate hospital infection control management.
  Accepted: January 2002
  Published: Abels et al., Journal of Clinical Microbiology 2001;39(9):3135-9
- Cand.med.: Eva Victoria Grams
  Titles of thesis: Nachweis des Humanen Herpesvirus 6 in Blutproben von Transplantationspatienten.
  Accepted: April 2002
- Cand.med.: Urs Stefan Hürlimann
  Title of thesis: „Grippe-Otitis“ - PCR-Nachweis viraler Erreger.
  Tutor: Prof. Schmid, Otorhinolaryngol. Dept., University Hospital Zurich
  Accepted: 2003
- Cand.med.: Simone Schmid
  Title of thesis: Parvovirus B19 Nachweis in Blut und Synovialflüssigkeit von Patienten der Rheumatologie (temptative)
  Tutor: Prof. Michel, Rheumatology Dept. University Hospital Zurich
  Status: On-going
- Cand.med.: Philipp Grob
  Title of thesis: Nachweis von respiratorischen Viren mit der Polymeraseketten-reaktion in Lungentransplantationspatienten.
  Status: On-going

The following FAMH candidates were trained in viral diagnostics in the diagnostic laboratories of the institute:

- Dr. Angelika Ströhle - April. 2000
- Dr. Simone Brunner January 2000
- Dr. Frantiska Palicova April - June 2000
- Dr. Susanne Abels March 2001 - December 2002
- Dr. Martin Risch October - December 2002
- Dr. Philipp Bosshard April - June 2003
- Dr. Sabine Berchtold September - November 2003
- Dr. med. & dipl. microbiol. Imeri Fatime December 2004

Fatime Imeri was the 20th FAMH candidate ever trained in the Institute since 1992!

8.5 Summary of Strengths and Weaknesses in the Areas of Services

Strength	**Measures Needed**
1. Monopole situation: Unique institution in the region offering cell cultures for broad-spectrum detection of infectious virus.	Stick to it!!!
2. All 3 approaches for virus detection (cell culture isolation, molecular diagnostics & serology) being performed in a single institute.	Stick to it! To the benefit of the patient.
3. Accreditation of diagnostic activities.	Stick to it.
4. Well educated and trained staff, highly motivated. Stable team.	Take care of it!

Weaknesses	**Measures Needed**
1. Fractionation of virology and virus diagnostics into different institutions of the University in the immediate neighbourhood.	Unite similar activities in a single institution (top-down governmental decision).
2. High degree of dependence from ONE customer (University Hospital ZH)	Take care of it (strategic requirement)!!!
3. Departmental border between the Institute of Medical Virology (Education Dept.) and the University Hospital(s) (Public Health Dept.) causing frictions on several levels.	Eliminate administrative hurdles, especially in the finance section. (Ev. move the institute to the Public Health Dept.). (Top-down governmental decisions).
4. Low involvement in clinical research due to point 2 (and maybe to the fact that the institute is run by a natural scientist)	
5. No 24-hours a day / 7 days per week service (Institute of the university!).	If really needed: Increase number of staff involved in diagnostics.
6. Diversity of electronic data handling systems within in the University Hospital and in the two microbiology institutes. Lack of on-line reporting to external customers.	Unify the electronic data handling systems. Connect the University institutes to the hospital system (top-down governmental decisions).
7. Low number of academics. Results in low power for test development and low involvement in clinical studies	Increase the number of academics (by 1-2)
8. Low number of technicians: Capacity limiting introduction of new tests.	Increase the number of technicians (by 1-2)
9. We depend on qualified medical technicians but do not offer positions for professional education for young adults.	Create 2-4 positions for professional education of medical technicians together with the Institute of Medical Microbiology

Strengths

The strengths of the Diagnostics Department are the cutting edge technology for virus diagnostics by means of PCR, light cycle, and other molecular approaches. It was a focus of the Institute to anticipate potential emerging viral problems or demands. Therefore, the IMV was prepared for Poxvirus diagnostics, SARS virus outbreak, Norwark virus (Norovirus) outbreak, etc. The Institute established novel virus diagnostics procedures in close collaboration with other institutions in Switzerland or other European countries. The chief of diagnostics participated in several quality control European evaluation procedures always with very good results. Furthermore, he participated in several international scientific and applied diagnostics meetings on virology in various European countries.

The Institute underwent the procedure of getting the status of High Quality Control (Akkreditierung) together with the IMM. It has maintained this quality standard throughout the years.

This is a strong but was also a heavy burden on the personnel as well as the budget because maintenance of this status requires enormous training of the personnel and surveillance of all SOP's (Standard Operating Procedures) as well as equipment.

The institute managed to acquire a double-door autoclave directly connected to the high-containment facility (P3). On the basis of this only minor technical measures are required for the Institute to become prepared for bioterrorism. Indeed the Institute has been selected for UBL center for the western part of Switzerland for bioterroristic attacks.

The leader of Diagnostics, W.B., has not only been evaluated for quality control, but has been appointed by the Swiss Authorities to evaluate himself the Diagnostics Department at St. Gallen supporting how well he is respected for his qualification by his colleagues and the Swiss Administration.

Weaknesses

The Diagnostic Department is tightly squeezed within very limited space in an old building. Furthermore, 1 1/2 floors have recently been taken away from the IMV and attributed to the Institute of Medical Microbiology (IMM). P3 facilities of the NZR were originally planned for 5 years only and then transfer to another location. This did not materialize. Furthermore, modern laboratories within the "Robinson" were planned in detail before my arrival in Zurich (1993). These plans were constantly changed and modified – and seem to have been given up now.

8.6 Research at the Diagnostic Unit

Scientists/academics of the diagnostic section

The academics/scientists of the diagnostic team don't do any basic research. They are absorbed by evaluation and validation of new diagnostic tests and are permanently involved in various clinical studies.

Development of new tests is done either on our own, or in the frame of medical theses, or in collaboration with colleagues from other institutions.

Clinical studies are usually under the control of the colleagues of the University Hospital. The goal of these studies usually is given by medical rational out of our reach, but diagnostics always plays a major part in it.

The medical theses tutored by the diagnostic academics are listed in the diagnostic section (see §8.4).

The type of test development and clinical studies is best reflected in the publications with PD Dr. W. Bossart as coauthor originating from these activities in the years 2000 - 2004:

- Zingg W, Colombo C, Jucker T, Bossart W, Ruef C. Impact of an outbreack of norovirus infection on hospital resources. Infect Control Hosp Epidemiol 2005;26(3):263-7.
- Linder T, Bossart W, Bodmer D. Bell's palsy and Herpes simplex virus: fact or mystery? Otol Neurotol 2005;26(1):109-13.
- Hatt JM, Grest P, Posthaus H, Bossart W. Serologic survey in a colony of captive common marmosets (*Callithrix jacchus*) after infection with herpes simplex type 1-like virus. Journal of Zoo and Wildlife Medicine 2004;35(3):387-390.
- Gessler P, Bischoff GA, Wiegand D, Essers B, Bossart W. Cytomegalovirus-associated necrotizing enterocolitis in a preterm twin after breastfeeding. Journal of Perinatology 2004;24(2):124-6.
- Baumann D, Pusterla N, Peter O, Grimm F, Fournier PE, Schär G, Bossart W, Lutz H, Weber R. Fieber nach Zeckenstich: Klinik und Diagnostik von akuten Zeckenstich-assoziierten Infektionskrankheiten in der Nordostschweiz. Deutsche Medizinische Wochenschrift 2003;128:1042-1047.
- Steiner F, Zucol F, Berger Ch, Fischer JE, Bossart W, Altwegg M, Nadal D. Use of simple heuristics to target macrolide prescription in children with community-acquired pneumonia". Archives of Pediatrics & Adolescent Medicine 2002;156:1005-8.
- Kronenberg A, Schupbach R, Schuknecht B. Bossart W, Weber R, Gilden DH, Speck RF. Multifocal vasculopathy due to Varicella-Zoster Virus (VZV): Serial analysis ov VZV DNA and intrathecal synthesis of VZV antibody in cerebrospinal fluid. Clinical Infectious Diseases 2002;35:330-3.
- Fehr T, Bossart W, Wahl C, Binswanger U. Disseminated varicella infection in adult renal allograft recipients: four cases and a review of the literature. Transplantation 2002(Feb 27);73(4):608-11.
- Kronenberg A, Seebach JD, Bossart W, Weber R. Polyclonal expansion of large granular lymphocytes during an acute cytomegalovirus primoinfection in a HIV-positive patient on antiretroviral therapy. Clinical Infectious Diseases 2001; 33(5):E34-6.
- Weber R, Bossart W, Cone R, Luethy R, Moelling K. Phase I clinical trial with HIV-1 gp160 plasmid vaccine in HIV-1 infected asymptomatic subjects. European Journal of Clinical Microbiology and Infectious Diseases 2001; 20:800-3.
- Abels S, Nadal D, Stroehle A, Bossart W. Reliable detection of Respiratory Syncytial Virus (RSV) infection in children for adequate hospital infection control management. Journal of Clinical Microbiology 2001;39(9):3135-9.
- Berger C, Day P, Meier G, Zingg W, Bossart W, Nadal D. Dynamics of Epstein-Barr virus DNA levels in serum during EBV-related disease. Journal of Medical Virology 2001;64(8):505-12.
- Nadal D, Bossart W, Zucol F, Steiner F, Berger C Lips U, Altwegg M. Community-Acquired Pneumonia in Children due to Mycoplasma pneumoniae: Diagnostic Performance of a seminested 16S rDNA-PCR. Diagnostic Microbiology and Infectious Disease 2001 Jan;39(1):15-9.
- Sauvant G, Bossart W, Kurrer M, Follath F (2000). [Diagnosis and course of myocarditis: a survey in the medical clinics of Zurich University Hospital 1980 to 1998]. Schweizerische Medizinische Wochenschrift 2000 Sep 9;130 (36):1265-71. German.
- Hardegger D, Nadal D, Bossart W, Altwegg M, Dutly F. Rapid detection of Mycoplasma pneumoniae in clinical samples by real-time PCR. Journal of Microbiological Methods 2000 Jun;41(1):45-51.

9 A Look Towards the Future of the Institute of Medical Virology as a Whole: Future Perspectives (by K. Moelling)

- Anstehende grössere strategische Entscheide / Veränderungen (Rücktritte / Berufungen, Neuausrichtung von Lehrstühlen, neue Schwerpunkte, mögliche Einführung neuer Studiengänge und -abschlüsse usw.)
- I will retire as requested by my contract in August 2008. I encourage some of my coworkers to plan their future and to leave by that date. All PhD students will have terminated by that date. Continuation should occur and the Diagnostics Department under the guidance of PD Dr. W. Bossart who is very highly qualified, well integrated in the University, independent and highly appreciated by all colleagues of the Medical Faculty. His qualification can be estimated by the fact that he was appointed to analyse the quality of viral diagnostics in St. Gallen appointed by the Bundesamt für Messwesen for QC. I highly recommend that he should continue until his retirement in the year 2014.
- Furthermore an unlimited contract has PD Dr. J. Pavlovic, who fulfils many functions required for the Institute such as biosafety, radioactivity, animal facilities: He is also in charge of large equipment and computers for decisions on renewal or repair.

  As far as teaching obligations are concerned, almost all of them are undergoing structural changes in the year of 2005 as mentioned above on the individual chapters. All these changes take place within the new concepts of the Medical Faculty, the University, and the ETH.

  A major change is to be expected in the Department of Electron Microscopy (EMZ) where the leader (Dr. Bächi) has retired in 2005 and an interims leader has been appointed for the next two years (Dr. Höcheli). A structure committee has been founded under guidance of Prof. Grosscurt who evaluates all electromicroscopy locations in Zurich to develop a more unified structure. The infrastructure of the EMZ was extremely valuable for the IMV and it will be a big loss if the EMZ will be translocated to another location. It will probably be combined with the Department of Anatomy and the Faculty for Mathematics and Natural Sciences (MN Faculty) even though the EMZ is much bigger and better equipped. In November 2005 I insisted that no positions of the EMZ should be transferred to thee Department of Anatomy and that in 2008 should the new Head of Virology. Presently we are using more than 15 microscopes for training of the medical students, the EM form 50 diagnostics per year and the laser-microscope as well as time laps for research purposes.

  Recently major decisions have been taken about various individual viral diagnostics activities associated with structures outside of the IMV such as diagnostics of hepatitis viruses, childhood related viruses, and HIV (NZR).

  Changes in space and laboratory locations will take place within the near future. This depends on projects by the Institute of Medical Microbiology. This will affect several laboratories.

  Furthermore, changes for the Library are expected. The library was shared by the IMV and IMM and was gradly reduced with the arrival of Prof. E.G. Böttger and presently depends largely on personal subscriptions by Karin Moelling. Thus, the library will not be maintained in its present form after 2008.

  As future perspective I envisage that my position should again be filled by appointing a successor. The virology diagnostics of the University should be combined from the various institutes to the IMV under such a leader. The new professor should be appointed because virology was severely underestimated for more than one generation. It has not only attracted attention since HIV emerged, but also with SARS outbreaks, Noro outbreaks, and upcoming threats with Influenza and bioterrorism. Also modern gene therapy is mainly based on viral vectors and will increase, which requires exercise and training.

  For public health concerns, the BAG, the clinical director of the USZ, the press including newspapers and TV, travel agencies, and even diagnostics companies have been contacting

the IMV for expert advice. Furthermore, gene therapy with viral vectors will increase in the future and may require expertise from the local virologies.

The space situation should be improved, translation research is increasing also in respect of gene therapy involving basic research and clinical research. The need for good translationary research was very obvious throughout our collaboration with members of the medical faculty including clinical trials.

The high quality diagnostics should be maintained not only for the benefit of patients but also for the hospital organization. A striking example was the diagnostics of SARS within 24 hours instead of 6 days as suggested by the BAG, which would have been an extreme burden for the hospital personnel. Also the Noro outbreak in 2004/2005 was totally dependent on our diagnostics, because we were the only diagnostics with this test established. We have now established H5N1 Influenza diagnostics in case it is required. In the future most diagnostics approaches will be performed by PCR, which we have already introduced for many viruses so far.

10 From the Unit to the Peer Reviewers: Questions, Tasks, Possible Problem Areas for Particular Scrutiny

> Dieses Kapitel wird i.d.R. den Experten anlässlich der Site Visit nochmals speziell in Erinnerung gerufen bzw. erneut vorgelegt.

- The IMV should be maintained as an individual institute. It has to be bigger if the Virology Diagnostics ongoing in Zurich is centralized. This should be the case. Since 20 years a new building comprising Virology and Microbiology, high containment laboratories and other facilities has been planned but never materialized. Such an institute would be adequate for the field of virology in the future.
- The Diagnostics of Virology is scattered among several institutes (Children Hospital, Clinical Immunology, Clinical Chemistry, Dermatology, HIV internal medicine, and NZR (National Center for Retrovirology). This should be focussed. The new structural committed for planning the future of the IMV should not perpetuate particular interests of the committed members such as viral diagnostics at numerous different places in other institutions. The IMV e.g. does not perform any diagnostics on hepatitis viruses, which simply does not make sense. Such a structure should not be maintained.
- The importance of Virology is beyond question. Infectious diseases have been neglected for almost a generation but are of increasing importance. Examples are HIV, SARS, and Norovirus outbreaks, as well as the constant threat of influenza epi-or pandemics based on newly emerging strains in birds in Asia. Another threat comes from viruses involved in bioterrorism. For this purpose the IMV has overtaken the responsibility for the eastern part of Switzerland. Interesting new approaches to Virology are reverse genetics, anti-viral mechanisms and mechanisms of viral antidefense mechanisms, as well as gene therapy.
- The NZR (National Center for Retrovirology) was separated from the IMV (a politically very unfortunate decision and in contrast to a written promise for my appointment as Head of IMV in Zurich in 1993). Destruction of my main research focus and ongoing political controversies resulted. Very counter-productive were the consequences for my research. The same pattern for appointments of university professors have since been repeated several times and ended with demission of the newly appointed colleagues (quotation: “a university does not have to fulfil promises” (Berufungszusagen)).
- The IMV is a contact point at the border between basic research and medicine. This has many advantages for both disciplines. However, this was also the basis for severe problems and controversies. This was especially the case in the interaction within the Department of Dermatology, headed by the Dean of the Medical Faculty with whom we performed a clinical trial. Four follow-up grant proposals were rejected because of political reasons even though I was not part of it.

- Future appointments of Professors should involve an advisor (or coach) for newcomers for better understanding of local habits, networks, sensitivities, and potential counteractions. This could help to avoid continued scandals published in the press about the Medical Faculty.
- I will retire as the Director of the IMV by the end of 2008. The continuation for the Diagnostics Department is guaranteed after this for about 6 more years through PD. Dr. Bossart. Furthermore, the most experienced senior group leader, PD Dr. Pavlovic, will be able to uphold his research as well as the complex infrastructure of the IMV. A new director will have to decide on the future of the other coworkers, following the regular cancellation period.
- The administration, which was shared by IMV and IMM will change because of retirement of the Head of Administration.
- The future of the NZR and EMZ has to be decided upon. The Natural Sciences Faculty (Department of Anatomy) is keen on getting more influence and reduction of the EMZ. The new Head of Virology has to be included in a rotating directorship. I requested this in written in the proposal.
- Pro and contra could be discussed for the kind of scientific background of a new director, MD or natural scientist.
- The major focus of research of the IMV could have been more on viruses and perhaps less on cancer, even though my cancer research was based on viral oncogenes. This was a consequence of the history during the initial phase of my Directorship and inhibition of all my HIV research. This was a severe loss for others and me.
- MDs have a different approach to research. PhDs may be helpful for a better scientific culture at the University Hospital.
- The future of the ETH will focus more on biological sciences as integral part of the technology program, e.g. Biotech. This is a good chance of the University for collaborations.
- It may be worth considering the maintenance of students exchange programs in a more formalized way with other Universities .My interaction with the two Universities in Berlin were very beneficial for the Institute to give it a more international standard. This program was maintained for 10 years without any formalities and proved to be extremely successful for recruitment of good students.
- Exchange programs with other countries may be worth considering. At the PhD student and postdoc level international collaborations have taken place, which is beneficial but also sometimes involves an additional level of complication. The IMV has been very international, which may be due to interest of the young generation in the field.
- The University has a technology transfer office, Unitectra, for biotechnology applications. The patent regulations have recently been defined for university teachers and inventors. There is a general consensus that technology transfer should be emphasized. Consequently patents are being filed. However, researchers very often lack the knowledge about the workload involved and the costs required. Harmonization of the rules for clinical trials with Europe have led to the consequence, that it is almost impossible to develop preclinical results through further steps. Interaction with Swissmedic has been positive, however, vaccine or gene therapy developments have become almost impossible for legal, technical, and financial reasons. This is in sharp conflict with the number of grant applications in which vectors or viruses are proposed to be developed for gene therapy. It is short-sighted by the granting organizations to ask for patent-filing. There are not enough support and resources to persue this. Unitectra does not help in partnering – in contrast e.g. to the technology transfer office of the Max-Planck Society (Garching Innnovations), which served as a model for Unitectra. We have invested time, money, efforts to file patents and drive projects into application. This was probably not a success.

- The publication policy for co-authors should be the request for a continuous contribution until a paper is accepted, not based on contributions, which date seven years back with no follow-up contributions. A new publication regulation should be set up.
- It should not be allowed to put pressure on a Full Professor for six years with the threat that she will not get renewal of her contract. From December 1999 till March 2005 I worked without a contract renewal. My contract ended 1998 and a provisional extension ended in December 1999. Because of this upcoming Evaluation I received a new appointment beginning of March of 2005. This contract renewal clearly stated, that I was under observation and therefore without a contract.
- The University appointed an Ombudsman. Based on my experience, I would like to suggest that a decision-making process on co-authorship should not last longer than a given period e.g. four weeks. It is impossible to follow restrictions by the Ombudsman not to publish a paper until a decision is made, which takes many months.

Part B: Brief Portrait of the Research Staff of the Institute of Medical Virology

11 Prof. Dr. K. Mölling

11.1 Research 2000 – 2004

We have long-standing experience using DNA coding for antigens as vaccines against viruses and cancer and DNA coding for cytokines as adjuvant or therapeutics (1). We have tested gag-pol-rev DNA as vaccine against HIV-1 in 4 patients at the USZ, whereby the GMP-DNA and the trial was organized by a US company, Apollon, where K. Moelling was a part-time employee as Head of Research before she came to Zurich. K. Moelling coordinated this clinical trial at the USZ. We followed the patients for safety parameters especially anti-DNA antibodies for almost a year and obtained good safety results, which were published (2). No efficacy was detectable, however.

We used tumor-associated antigens coded for by DNA in mouse models and observed that a combination of DNA with xenopeptides was very efficient (3). The xenopeptides comprised CTL epitopes of about 9 amino acids with 1 mutation. Dr. J.D. Wolchok at the Sloan Kettering Institute, New York, and colleagues, have based a clinical trial on our publication and reported on some success at a meeting in Glasgow (2002).

We have analyzed about a dozen of genes coding for melanoma-associated antigens, cytokines or suicide genes, such as gp100, IL-12, IL-2, GM-CSF, B7.1, B7.2, IFN-γ, IP-10, VEGFEC (vascular endothelial growth factor extra-cellular domain) or HSV-TK (4, 5, 6 and unpublished). IL-12 DNA was far more superior against several tumor models in mice than any other agent tested.

Therefore, we focused our attention on IL-12 DNA as gene medicine. IL-12 is a heterodimeric cytokine composed of a p35 and a p40 subunit. IL-12 promotes cell-mediated immunity. IL-12 stimulates production of IFN-γ from T cells and NK cells. Studies in IL-12-deficient mice have demonstrated an essential role for IL-12 in the induction of Th1 responses, which are especially required for protection against intracellular microorganisms. IL-12 is not only involved in induction but also in the maintenance of Th1 responses.

Production of IL-12 by activated macrophages and dendritic cells (DCs) results in secretion of a 10-1000-fold excess of free monomeric and homodimeric p40 relative to heterodimeric IL-12. The activities of IL-12 are mediated by a high-affinity receptor composed of two subunits expressed on NK and T cells. Signaling occurs via STAT4 by transcription activation of responsive genes in NK and T cells.

We were able to attribute the anti-tumor effect of IL-12 DNA by experimental analysis to anti-angiogenesis (collaboration with Drs. A. Albini and D.M. Noonan, Genova) (7). The contribution of natural killer (NK) cells has been described in the literature and we demonstrated their effect by depletion with monoclonal antibodies (4). We used human and murine IL-12 in mice and observed a dramatic effect of expression up to 60 days only with the murine not human IL-12 DNA (5). This suggests that an autocrine effect may be involved in the homologous species. The mechanism has not been studied further.

We furthermore observed not only a therapeutic effect of IL-12 DNA in a local treatment of cancers but also a prevention of the establishment of metastases. This was totally unexpected. When IL-12-encoding DNA was injected into mice nine days before tumor cell challenge, no metastases were formed (5). Apparently the treatment prevented colonization of the tumor cells to the lung. This is a phenomenon, which has not been described or explained, yet.

Furthermore, we tested another animal model besides mice. We chose among several different ones gray horses, which naturally develop malignant melanomas. Seven horses were

treated by local injection of human IL-12 DNA. After a few injections the tumors reduced in size and consistency. The animal keepers were able to notice this immediately. All nine tumors tested in seven horses showed strong reduction in size (8). We published and submitted these data to the SKBS/Swissmedic and received approval for an investigator (K. Moelling) -driven Phase I/II Clinical Trial on late stage cancer patients (1999), without support from a company.

IL-12 DNA may prove useful for tuberculosis or other parasites also (9) and may be considered as a component in a combination therapy in the future.

K. Moelling submitted the clinical protocol to SNF37 (1999-2002) and received a grant for financing part of the GMP-DNA production, which was performed by Berna Biotech AG together with the initial help of colleagues in the USA.

A clinical study has been performed by intratumoral injection of DNA encoding human IL-12 into 9 patients with late stage metastatic malignant melanoma, which showed clinical efficacy. K. Moelling was sponsor and scientific coordinator. The DNA was applied in cycles, three injections per cycle for up to 7 cycles. One cycle consisted of 3 injections, one per week and a subsequent rest of one week. Three therapy arms comprised low (2 mg), medium (4 mg), and high (10 to 20 mg) amounts of total DNA. The therapy was well tolerated. Three of nine patients experienced a clinical response: two of them stable diseases and one complete remission. One patient receiving a low dose of DNA experienced a long-lasting stabilization of the disease for more than 3 years, whereas the other two responders received high doses of DNA. All patients but one experienced a transient response at the intratumoral injection site. Immunohistochemical staining of sections from responders showed local reduction of angiogenesis and lymphocyte infiltrations. All patients, in particular the responders, exhibited an antigen-specific immune response against MAGE-1 and MART-1, which in some cases pre-existed. Biopsies of responders showed some increase in expression of IL-12, IP-10, and IFN-γ. Serum levels revealed fluctuations. The results show that intratumoral injection of DNA produced some beneficial clinical effect. DNA encoding a cytokine may be useful as a therapeutic or adjuvant against various human cancers (10).

Based on these results we have been encouraged by Swissmedic to continue with analysis of a higher number of patients. We are presently preparing a clinical trial with 21 more patients with late stage malignant melanoma in two clinical centers in Switzerland. The GMP DNA production is almost finished. The clinical protocol is presently being amended and it is envisaged to begin with the analysis of additional patients in 2006.

Furthermore, two compassionate trials are ongoing, which are based on scientific collaborations at the USZ and the Charité, Berlin. A collaboration with the National Cancer Institute (NCI), Bethesda, Maryland, USA, has been initiated with Dr. Jon M. Wiggington, as a scientific collaborator and a clinical investigator. We intend to use the IL-12 DNA possibly in combination with other factors in patients at the NCI.

Recently, the signal transduction protein B-Raf has been identified as a validated target in malignant melanoma cells. The applicant of this proposal (KM) discovered the Raf kinase in 1984 (11) as a serine-threonine protein kinase and this has been a major focus of basic research in the Institute of Medical Virology for the last 10 years (12), and literature quoted therein). An inhibitor against B-Raf developed by Bayer Co., which is in phase II Clinical Trial, will be studied at the NCI in a combination with IL-12 DNA.

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11.2 Signal Transduction: 20 Years Raf Kinase (1984-2004)

Retroviruses can replicate and cause diseases in animals and humans. Oncogenic retroviruses are normally defective in replication but code for viral oncogenes, which are derived from cellular proto-oncogenes. Many of these oncogenes represent signal transducers such as growth factors, growth factor receptors, membrane-associated tyrosine kinases, cellular serine/threonine kinases and transcription factors. In contrast to their cellular counterparts they are constitutively activated by mutations. Most of these oncogenes give their virus a selective advantage for increased proliferation. In 1982 we were able to isolate a monoclonal antibody against the viral structural protein Gag, which is fused in many cases to the oncogenic proteins. Many of these so-called Gag-onc fusion proteins were recognized by the monoclonal antibody against Gag, which allows the identification, localization, and isolation of half a dozen fusion proteins. Among these were Myc, Myb, ErbB, Fes, as well as Mil-Raf. In 1984 we identified Mil-Raf as an intracellular serine/threonine protein kinase, which was different from all the then known oncogenic tyrosine kinases. Since then Raf was a major focus of research in our laboratory.

On the occasion of a celebration of the Max-Planck-Institute of the Molecular Genetics in Berlin, a retrospective on 20 years of Raf kinase allowed us to summarize properties of the Raf kinase in 20 different models summarized in the Figure 1 (next page).

In the models the Ras-Raf kinase cascade is indicated by arrows representing the signal transduction pathway. (1) Shows an oncogenic retrovirus-derived protein kinase, which does not require receptor-mediated activation. (2) In the normal cellular situation ligand activation of an RTP leads to kinase cascade and cellular proliferation of a normal cell. (3) Shows a transient activation. (4) positive and negative feedback mechanism as indicated. (5) Depending on ligand and receptors signal transduction pathways can lead to a strong or weak response, which can lead to either differentiation or proliferation, depending on the background of the cell. (6) Growth factor stimulation by a cell bound ligand leads to a constitutive receptor activation and then to differentiation (drosophila eye). (7) Receptor stimulation from diverse inputs can be integrated for the cellular response. (8) There are several kinase cascades analogous to the Raf kinase cascade, which may be integrated for downstream responses. (9) At various levels of the cascade signaling can diverge. (10) Two different signaling pathways can cross talk and lead to diverse cellular responses depending on the ligands (as shown for Akt and Raf cross talk). (11) Scaffold proteins can help to keep components of the signal transduction cascade in close contacts. (12) A bypass of the downstream effector of Raf appears to be Rip-2. (13) Active quiescence kinases are known to be required for the maintenance of a non-proliferative state. Some of these are tumor suppressor kinases (such as Bcr). These kinases need to be inactivated in a proliferative

situation. (14) Receptor ligand interaction by cell-cell contact can lead to bidirectional signaling. (15) Antiproliferative signaling, and cellular junctions can be regulated by proteins containing PDZ domains, which function as blocks for signal transduction. (16) Epithelial cell layers and formation of polarized cells are also affected by PDZ proteins. Close contacts are required for spread of viruses. (17) Insulin stimulation leading to transmembrane glucose uptake can be mediated by a vesicular propeller-type scaffold protein, which can transport insulin-activated kinases. (18) The chemokine receptor CCR5 of HIV is regulated by a four-transmembrane protein JM4 in cellular trafficking. (19) Inside out signaling can induce integrin (20) Asymmetric Wnt- and growth factor- signaling in stem cells.

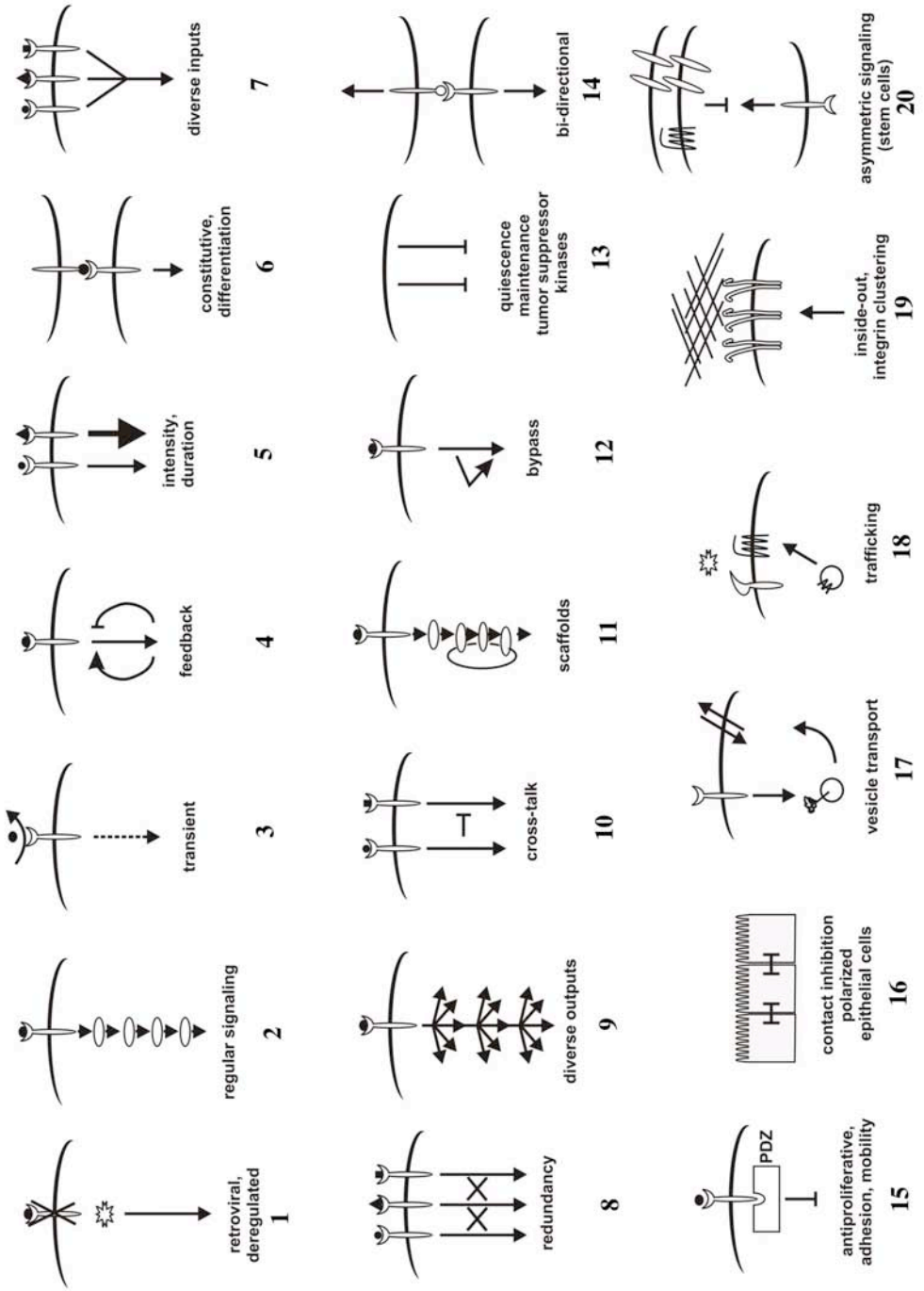


Figure 1: Overview of signal transduction pathways

The Raf kinase family. We have originally discovered the protein kinase Mil/Raf as oncogene of avian and murine retroviruses (1). It was distinct from the then known oncogenic kinases, as it was not a tyrosine kinase but a cytoplasmic serine/threonine kinase. It has been an enigma for many years, why the cellular Raf exhibited a higher specific enzyme activity than the viral fusion protein Gag-Mil/Raf, which is strongly expressed but rather inactive (2). This was surprising because retroviral oncogenes are known to function by growth-promotion. The answer comes from the fact that Raf can induce growth but when highly activated it can activate cell-cycle inhibitors causing cellular senescence or death depending on the cellular background.

The family of Raf kinases contains three mammalian isoforms (Fig. 2). Raf-1 is ubiquitously expressed, A-Raf is expressed in kidney and liver. B-Raf was originally found in neuronal tissue but is now found expressed at different levels in many tissues. Very recently, it has been shown that B-Raf is activated by somatic mutations in several types of human tumours, mainly in malignant melanomas.

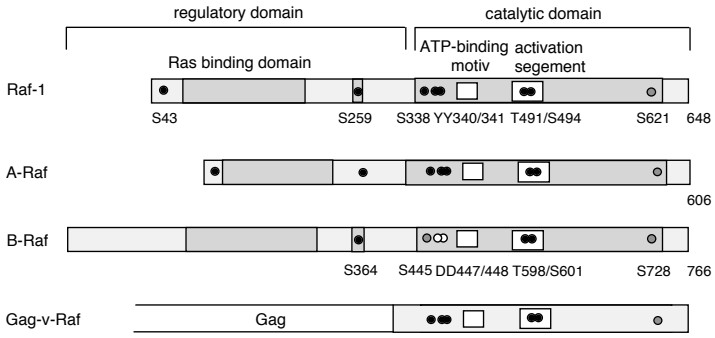
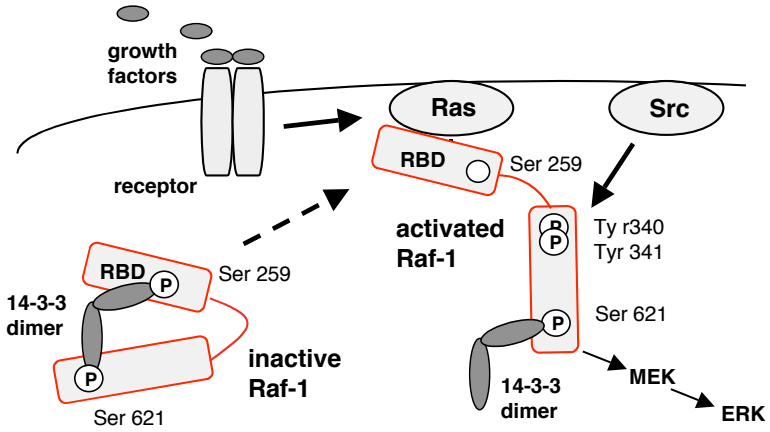


Figure 2: Mammalian isoforms of the Raf protein kinase family. Black dots represent regulatory phosphorylation sites, white dots represent negatively charged residues mimicking phosphorylation.

The Raf/MEK/ERK pathway. Genetic and biochemical analyses in the nematode C. *elegans*, the fruit fly *D*. *melanogaster*, and in mammalian cells have established that Raf connects signals from the Ras proto-oncogene to the MAP kinase cascade. The best-characterised immediate downstream substrates of activated Raf is the kinase MEK, which becomes activated upon phosphorylation by Raf. Activated MEK in turn phosphorylates and activates the p42 and p44 MAP kinases. Following this, MAP translocates from the cytoplasm to the nucleus, where it phosphorylates and regulates the activity of transcription factors, leading to multiple changes of the gene transcription pattern, and consequently to cellular responses such as cell cycle re-entry or differentiation. We characterized a positive feedback loop, which activates Raf via MEK independently of Ras and Src, the main stimulators of Raf-1. This positive feedback allows a fast signal amplification and prolonged activation of Raf (3). Furthermore we showed that in mitotic cells a Ras-independent mechanism resulted in a cytoplasmic and not membrane-associated activated Raf, which did not signal via the MEK-ERK pathway (4).

Mechanisms of Raf regulation. In unstimulated cells Raf is fixed in an inactive form by a 14-3-3-dimer binding to the N - and the C - terminus of Raf (Fig. 3). Ligand dependent stimulation of a receptor tyrosine kinase (RTK) leads to activation of Ras via the adaptor protein Grb2 and the nucleotide exchange factor SOS. In turn activated Ras recruits Raf to the plasma membrane thereby preventing further binding of 14-3-3 to the N - terminus of Raf. Additional steps such as phosphorylation of tyrosine residues by Src fully activate Raf kinase activity. Activated Raf stimulates a protein kinase cascade including the kinases MEK and ERK.

Figure 3: Mechanism of Raf activation.



Recently, we have characterized Raf and identified some of the co-precipitating proteins such as Hsp90, Jun, 14-3-3, Ras and pp34, a phosphoprotein in proliferating but not in mitotic cells (5-7). Complex-formation of pp34 and Raf depended on serum stimulation and altered during the course of cell-cycle progression (8). In a Drosophila eye developmental system we demonstrated that wild type Raf inhibited the formation of photoreceptor cells in a dose-dependent manner (9). Binding of the 14-3-3 protein to the N-terminus of Raf was shown to cover the S259 phosphorylation site which negatively regulates the Raf kinase. In Xenopus oocyte extracts as test system we could prove that a small Raf-derived peptide comprising S259 can inhibit Raf/MAPK signaling (10). Retroviral vectors were constructed and used as tools for stable insertion of genes. We demonstrate that a dominant negative mutant of Raf reduced malignant transformation of a tumor cell line but death of tumor cells occurred only when a second inhibitor, a dominant negative Myc, was involved (11). Inducible retroviral vectors, the so-called tet-off system, was established and used to induce expression of the constitutive active C-terminus of Raf. A subtractive hybridization was performed with mRNAs of induced and non-induced Raf-1 cells carrying the tet-Raf C-terminus. In collaboration with the Max-Planck-Institute for Molecular Genetics, Berlin, a robot-assisted analysis using nylon membranes was performed which led to 9216 transformants. Some of the strongly induced genes were further characterized. Among others were metalloproteinases, a chemo-attracting protein, Interferon-induced IP-10, and a tumor suppressor PAI-2 (12).

Signaling from transmembrane receptors does not only depend on ligand binding but also on intracellular scaffold molecules that might participate in receptor clustering. An important group of signaling molecules are PDZ domain containing proteins that bind to C-terminal sequences ending with a valine residue. In order to investigate some novel aspects of signal transduction from RTK we picked a representative of these receptors with a characteristic carboxyterminus (a valine) and performed a yeast-two-hybrid screen. This led to the identification of the protein AF-6 known as fusion partner in certain leukemias. The binding involved a PDZ domain, a characteristic 100 amino-acid domain with a conserved GLGF motif, which binds in a sequence-specific manner to a C-terminal target peptide (S/T X V). A degenerate PCR methodology allowed a mutational analysis of PDZ domains and characterization of their ligand peptide sequences (13). The AF-6 PDZ domain was found to interact with C-termini of several RTKs, e.g. Eph receptors. Eph receptors and AF-6 co-cluster at sites of cell-cell contact and at post-synaptic membranes of excitatory synapses in the hippocampus (14).

Cross-talk of signaling pathways. A main interest of the current work on Raf signaling is its role in complex biological systems. In addition to the Raf/MEK/ERK pathway there is a second major phosphorylation cascade important in proliferative and developmental decisions in multicellular eukaryotes, the phosphatidylinositol 3-kinase (PI3K)-Akt pathway. Responses to activation of this pathway depend on the cellular context, but commonly involve either cell proliferation or cell survival (where, in the absence of activation of this pathway, the cells would have entered an apoptotic program). Accordingly, Akt has been found to phosphorylate

and regulate several transcription factors and components of the apoptotic machinery. Again, the best understood regulatory mechanism for this pathway involves its activation downstream of transmembrane receptor tyrosine kinases. We have been able to demonstrate that the Raf/MEK/ERK pathway can be regulated by the PI3K-Akt-pathway (Fig. 4). Akt can phosphorylate S259 and physically interacts with Raf (15). We have analysed two different biological systems, mouse C2C12 skeletal muscle cells and human MCF-7 breast cancer cells (15, 16). MCF-7 cells unexpectedly showed an activated Raf pathway towards differentiation. It would have been expected that differentiation requires a shut-down of Raf and a subsequent stop of proliferation. This turned out to be the situation in a skeletal muscle differentiation system, where Raf needs to be shut off in differentiated cells. Further analyses revealed that the phenotypic modulation of vascular smooth muscle cells is also regulated by the Akt/Raf crosstalk (17). In this case Akt-dependent downregulation of Raf results in proliferation as is the case for MCF-7 cells. Coordination of the two pathways in a single cellular response depends on many parameters, such as cell-type, stage of differentiation, external stimuli, their dose, duration, combination, or growth conditions, e.g. cell-cell contacts, substrates or feeder layers, autocrine loops, etc (18). It is the goal to resolve these seemingly conflicting observations by analysing other differentiation systems and determine the principles behind them.

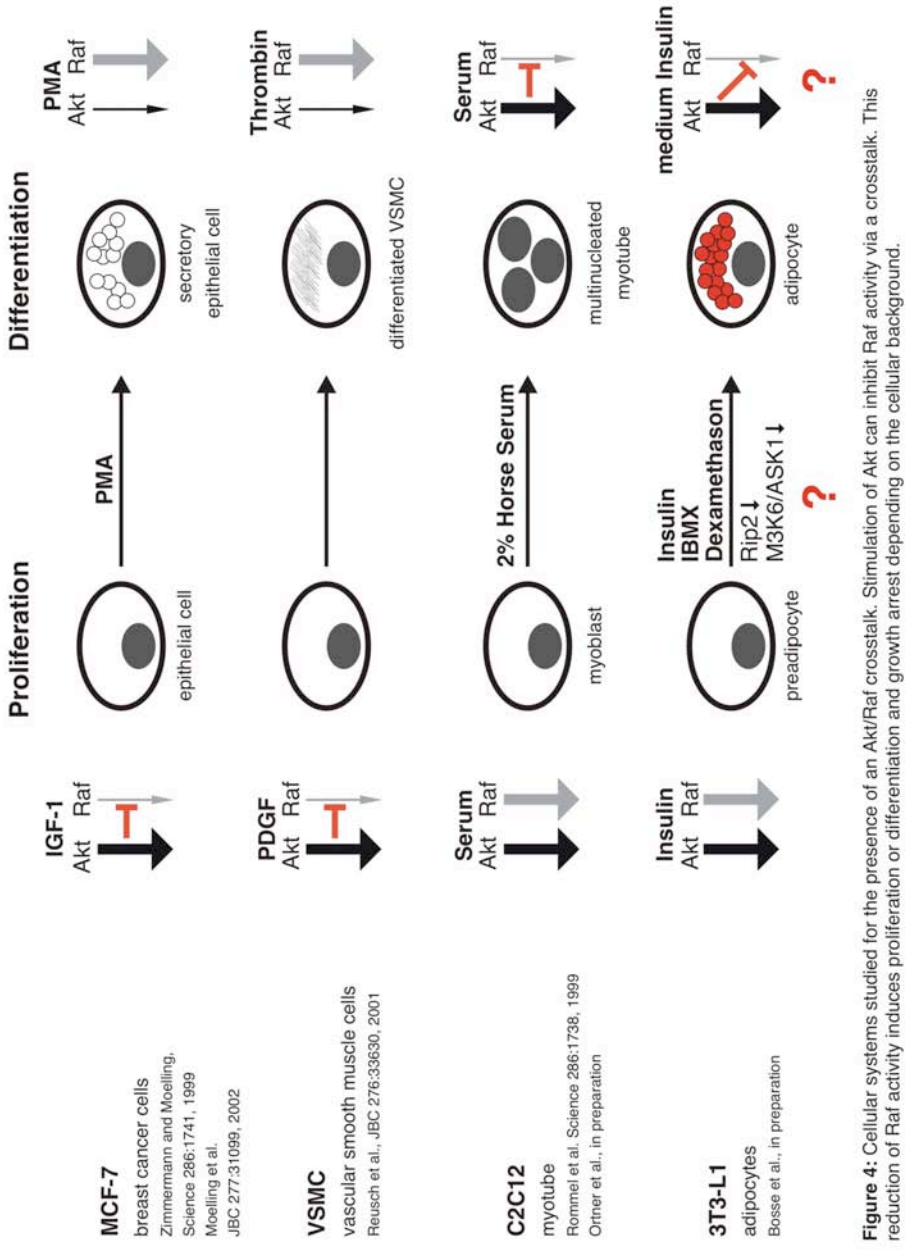


Figure 4: Cellular systems studied for the presence of an Akt/Raf crosstalk. Stimulation of Akt can inhibit Raf activity via a crosstalk. This reduction of Raf activity induces proliferation or differentiation and growth arrest depending on the cellular background.

Signaling network and antiproliferative signaling

We are presently extending our work on signal transduction by analyzing antiproliferative effects. These are of biological relevance in quiescent cells such as cells in epithelial monolayers. In such situations cell-cell contact is maintained and cells do neither proliferate nor differentiate. Only when they become individualized the cells start proliferating. These proteins involved in antiproliferative signaling are in several cases PDZ-proteins, such as CNK, ERBIN and AF-6.

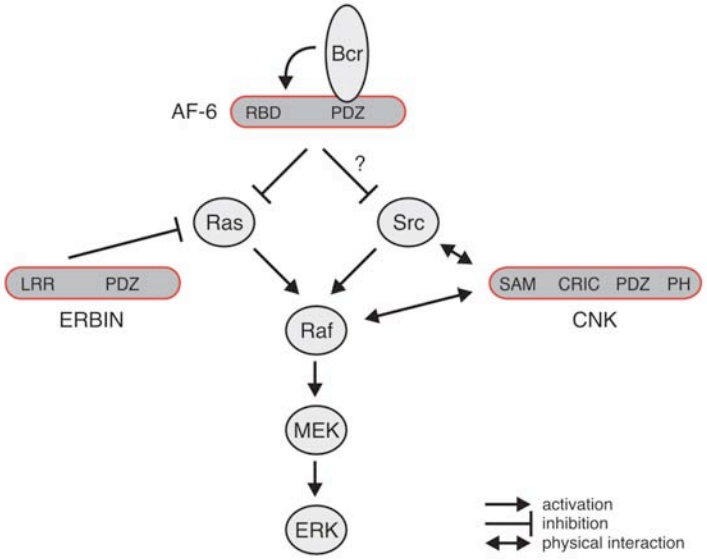


Figure 5: Regulation of Raf signaling by PDZ domain-containng proteins

The scheme summarizes the function of different PDZ domain-containing proteins on the regulation and activation of Raf signaling. AF-6 interacts with both of the Raf activators, Ras and Src, and thereby down-regulates their activity. AF-6 itself is activated by phosphorylation, e.g. by the Ser/Thr kinase Bcr. The scaffold CNK connects Src and Raf-1 and allows efficient Src-dependent activation of Src. ERBIN found as interaction partner of the receptor tyrosine kinase ErbB2 negatively regulates Ras via its leucine-rich region (LRR) domain. All these PDZ domain containing protein have in common that they lead to down-regulation of proliferative signaling or hold a cell in a quiescent state. RBD, Ras binding domain.

CNK was identified in the Drosophila system as connector enhancer of kinase suppressor of Ras and was described as interaction partner of D-Raf. We were able to demonstrate that the human homologue CNK1 connects Raf-1 and Src and regulates Src-dependent phosphorylation and activation of Raf-1 (19). CNK is a multidomain protein, which could be the basis for generating a large signal transduction complex. We identified the angiotensin II type 2 receptor as interaction partner of CNK (20). Using the yeast-two-hybrid system we identified several novel CNK1-interacting partners that are now under further investigation.

AF-6 has been recently characterized in epithelial cells with tight cell-cell junctions. Furthermore, a new isoform was cloned and analyzed. The siRNA-mediated knock-down of AF-6 leads to loss of cell contacts, increased mobility and an interesting phenotype with increased directionality as evidenced by time-lapse. The cells do require AF-6 for searching for contact partners (21).

AF-6 mediates not only RTK clustering but also binds to Ras thereby regulating the Raf/MEK/ERK pathway. The Ser/Thr kinase Bcr (break point cluster region) can induce downregulation of Ras signaling via AF-6. Bcr is active in quiescent cells and inactivated upon growth factor stimulation of the cell. Bcr phosphorylates AF-6, which allows binding of Bcr to the PDZ domain of AF-6 and increases the affinity of AF-6 for activated Ras. In turn, AF-6 competes with Raf for binding to Ras and reduces Ras/Raf signaling (22).

Bcr furthermore, turned out to be an inhibitor of the transcription factor β-catenin in the Wnt signal pathway. Upon growth factor stimulation or by means of Bcr-Abl in tumor cells Bcr

becomes phosphorylated and loses its inhibitory effect on β-catenin, thereby promoting transcription of genes such as c-myc and enhancing the proliferative activity of the cell. The inhibitor Gleevec, used for Chronic Myelogenous Leukaemia inhibits Bcr-Abl, which then allows Bcr to show its anti-proliferative effect, which increases the effect of the drug (23).

In cooperation with Hartmut Oschkinat, FMP Berlin, and Rudolf Volkmar-Engert, we screened a C-terminal peptide library for binding of selected PDZ domains. We identified several known and novel ligands for the PDZ domains of the two GTPase binding proteins Erbin, ErbB2 interacting protein, and AF-6, localised at cellular junctions (24, 25).

Recently we noticed that the c-Src protein has a C-terminal sequence, which is a putative PDZ-ligand domain. We noticed that various PDZ-proteins can regulate c-Src in a negative way by restricting the number of substrates for phosphorylation. This is in contrast to the properties of v-Src, which is very promiscuous. We are presently characterizing three PDZ-proteins for the putative role for Src regulation.

We have characterized various protein-protein interaction partners, which we identified by yeast-two-hybrid screen. One of them is the protein VLAK or ProF. This is a propeller-type protein, which binds two protein kinases, Akt and PKCzeta, preferentially in their activated states by insulin or growth factor stimulation. The propeller-type protein harbours a FYVE-domain, which targets the complex to vesicles. Upon insulin stimulation the whole structure translocates to the membrane of differentiated adipocytes for regulation of glucose uptake, which we demonstrated by siRNA-mediated knock-down. Furthermore, a targeting protein, v-SNARE, is part of the complex and mediates docking to t-SNARE proteins at the membrane (ongoing project, Thesis T. Fritzius).

We extended the study of signaling networks (Fig. 6) by characterization of a newly identified gene products, JM4, which binds to the CCR5 co-receptor of HIV-1 (26). In addition we described the interaction between CCR5 and the cytoskeletal protein α-catenin (27).

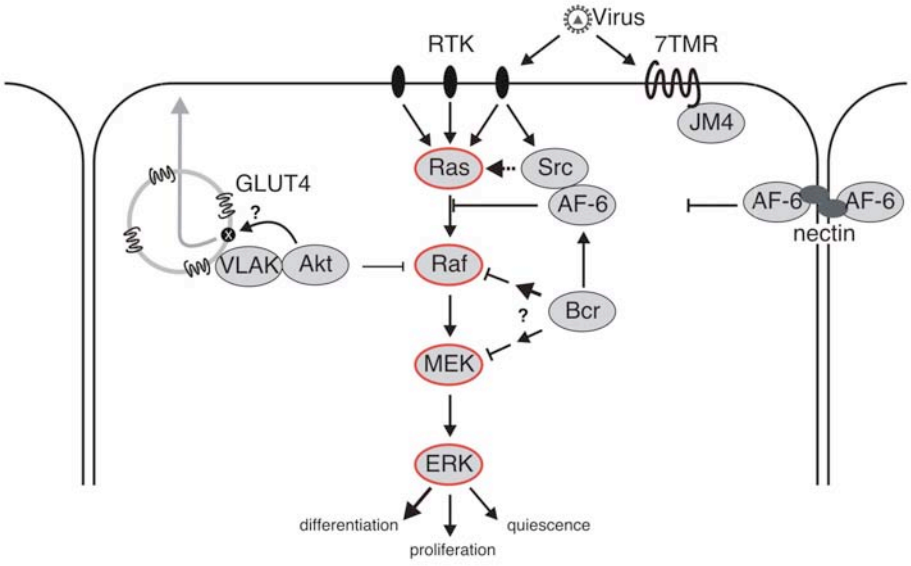


Figure 6: Signal transduction pathways investigated at the IMV

The IMV studies the Ras-Raf-MEK-ERK pathway and its negative regulation in differentiating or quiescent cells (antiproliferative signaling) by kinases such as Akt or Bcr. The antiproliferative signaling is mediated by PDZ-domain containing proteins such as AF-6, ERBIN or CNK. AF-6 is also involved in cell-cell contacts. Furthermore we study a vesicle-linked adapter protein, VLAK or ProF, which interacts with activated Akt and PKCz and regulates the insulin-dependent translocation of GLUT4 from internal vesicles to the plasmamembrane. Finally we are interested in the signaling during viral infections where we study JM4 binding to coreceptors for HIV.

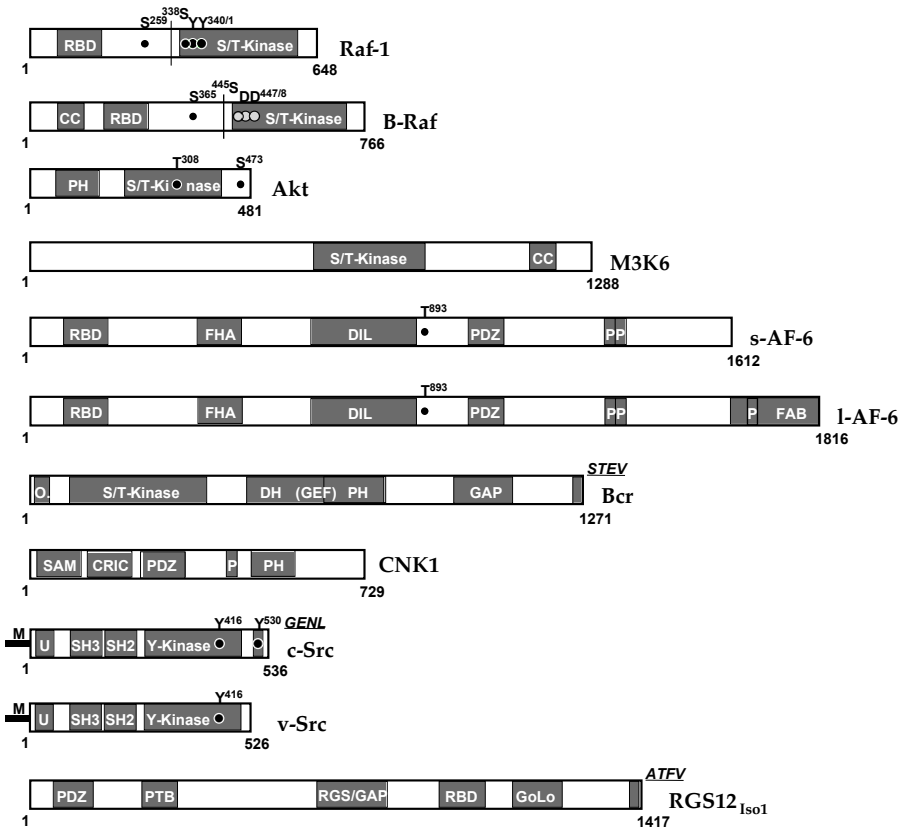


Figure 7: Domain structure of signaling proteins used at the IMV.

Abbreviations: CC = coiled coil domain, FAB = F-actin binding region, GoLo = GoLoco domain, M = myristoylation, o.= oligomerization domain, P = proline-rich region, PTB = phosphotyrosine binding domain, RBD = Ras and Rap binding domain, RGS = regulator of G protein signaling, GAP = GTPase activating protein, U = unique region (putative) PDZ-binding C-termini are in italic and undelined. Numbers indicate amino acid positions, specific phosphorylation sites and N- or C-termini. The schemes represent the human proteins except for chicken v-Src.

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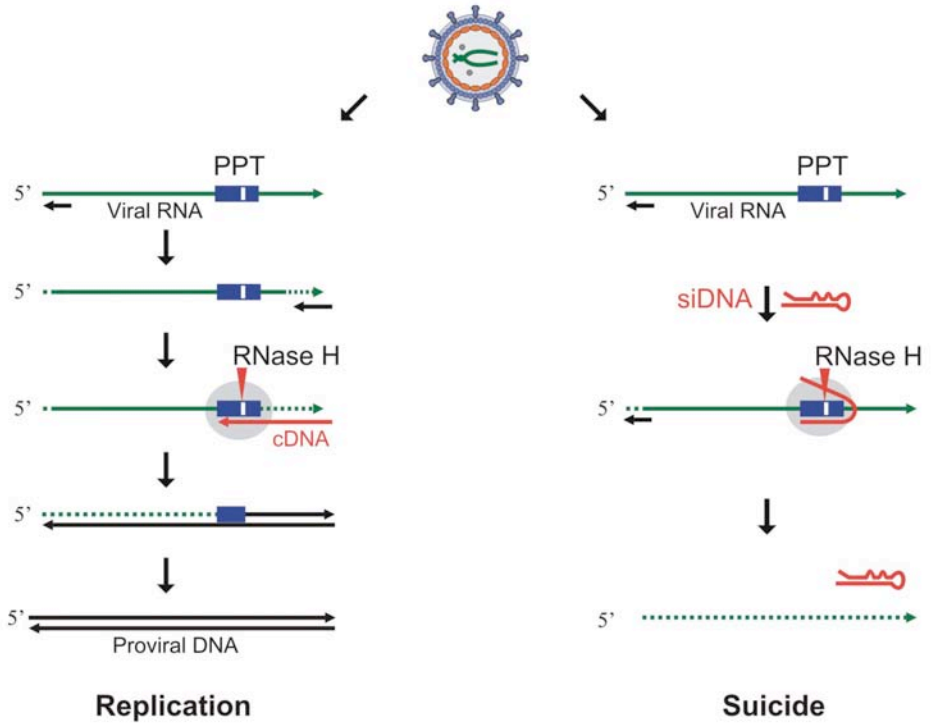
Direktorin: Prof. Dr. Karin Mölling

33 Years of RNase H

1972 – 2005

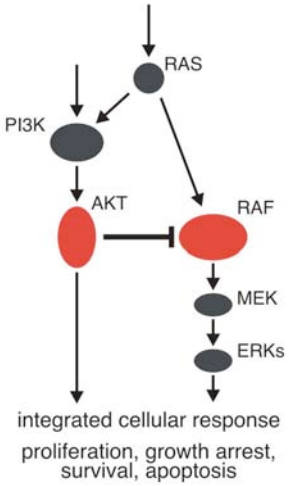
HIV-silencing by siDNA



see 11.5.2

11.2.1 Phosphorylation and Regulation of Raf by Akt (Protein Kinase B)

Science **286**, 1741-1744 (1999)
Science **286**, 1738-1741 (1999)



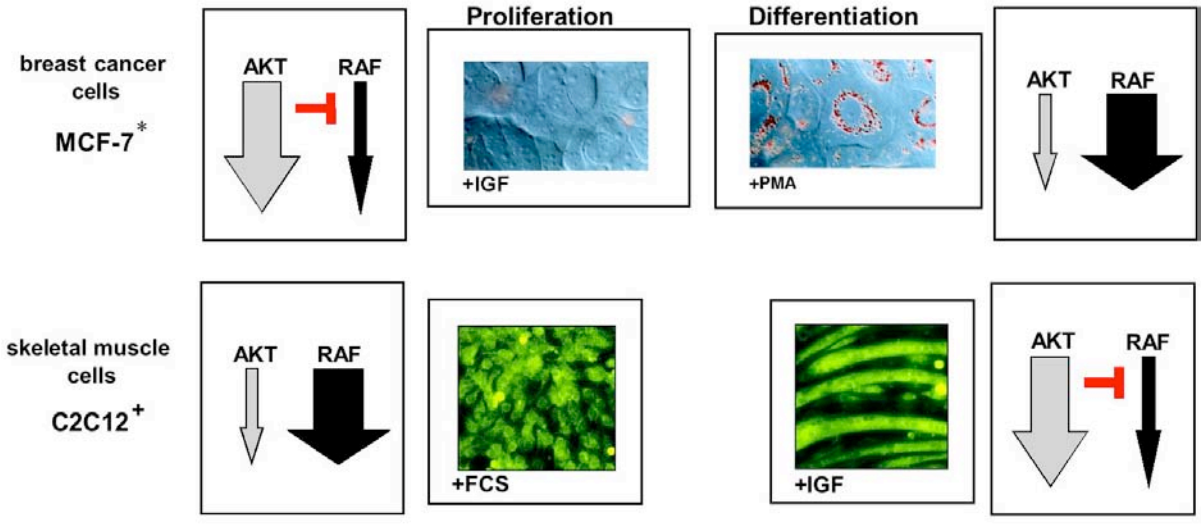
Sven Zimmermann and Karin Moelling*

Activation of the protein kinase Raf can lead to opposing cellular responses such as proliferation, growth arrest, apoptosis, or differentiation. Akt (protein kinase B), a member of a different signaling pathway that also regulates these responses, interacted with Raf and phosphorylated this protein at a highly conserved serine residue in its regulatory domain in vivo. This phosphorylation of Raf by Akt inhibited activation of the Raf-MEK-ERK signaling pathway and shifted the cellular response in a human breast cancer cell line from cell cycle arrest to proliferation. These observations provide a molecular basis for cross talk between two signaling pathways at the level of Raf and Akt.

Christian Rommel,[1] Brian A. Clarke,[1] Sven Zimmermann,[2] Lorna Nuñez,[1] Roni Rossman,[1] Kristina Reid,[1] Karin Moelling,[2] George D. Yancopoulos,[1]* David J. Glass[1]*

Extracellular signals often result in simultaneous activation of both the Raf-MEK-ERK and PI3K-Akt pathways (where ERK is extracellular-regulated kinase, MEK is mitogen-activated protein kinase or ERK kinase, and PI3K is phosphatidylinositol 3-kinase). However, these two signaling pathways were shown to exert opposing effects on muscle cell hypertrophy. Furthermore, the PI3K-Akt pathway was shown to inhibit the Raf-MEK-ERK pathway; this cross-regulation depended on the differentiation state of the cell: Akt activation inhibited the Raf-MEK-ERK pathway in differentiated myotubes, but not in their myoblast precursors. The stage-specific inhibitory action of Akt correlated with its stage-specific ability to form a complex with Raf, suggesting the existence of differentially expressed mediators of an inhibitory Akt-Raf complex.

Cross-talk of Akt and Raf, for details see next pages



- relative signal intensities decide on proliferation or differentiation
- cell type decides on the actual outcome

11.2.2 Regulation of Raf by Akt Controls Growth and Differentiation in Vascular Smooth Muscle Cells*

THE JOURNAL OF BIOLOGICAL CHEMISTRY Vol. 276, No. 36, Issue of September 7, pp. 33630–33637, 2001

H. Peter Reusch‡§, Sven Zimmermann§¶, Michael Schaefer_, Martin Paul, and Karin Moelling¶
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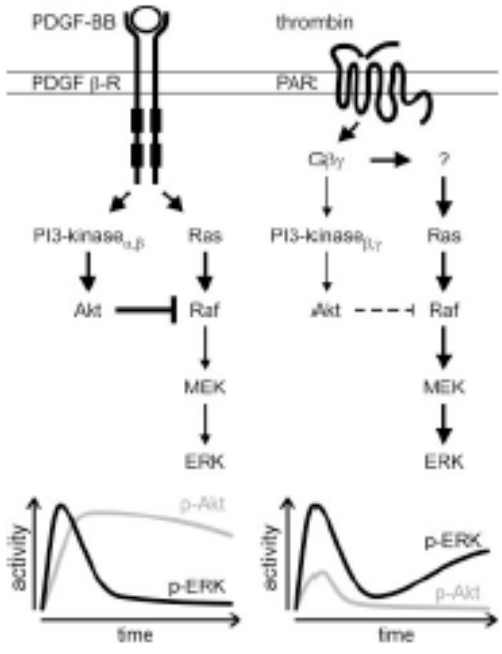
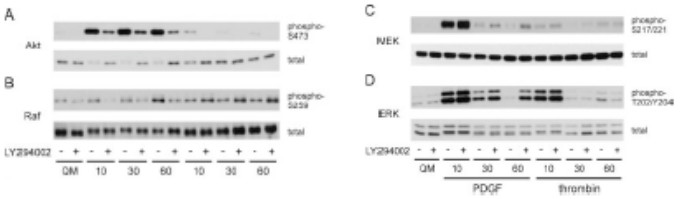
The stimulation of platelet-derived growth factor (PDGF) receptors shifts vascular smooth muscle (VSM) cells toward a more proliferative phenotype. Thrombin activates the same signaling cascades in VSM cells, namely the Ras/Raf/MEK/ERK and the phosphatidylinositol 3-kinase (PI 3-kinase)/Akt pathways. Nonetheless, thrombin was not mitogenic, but rather increased the expression of the smooth muscle-specific myosin heavy chain (SM-MHC) indicative of an *in vitro* re-differentiation of VSM cells. A more detailed analysis of the temporal pattern and relative signal intensities revealed marked differences. The strong and biphasic phosphorylation of ERK1/2 in response to thrombin correlated with its ability to increase the activity of the SM-MHC promoter whereas Akt was only partially and transiently phosphorylated. By contrast, PDGF, a potent mitogen in VSM cells, induced a short-lived ERK1/2 phosphorylation but a complete and sustained phosphorylation of Akt. The phosphorylated form of Akt physically interacted with Raf. Moreover, Akt phosphorylated Raf at Ser259, resulting in a reduced Raf kinase activity and a termination of MEK and ERK1/2 phosphorylation. Disruption of the PI 3-kinase signaling prevented the PDGF-induced Akt and Raf-Ser259 phosphorylation. Under these conditions, PDGF elicited a more sustained MEK and ERK phosphorylation and increased SM-MHC promoter activity. Consistently, in cells that express dominant negative Akt, PDGF increased SMMHC promoter activity. Furthermore, expression of constitutively active Akt blocked the thrombin-stimulated SM-MHC promoter activity. Thus, we present evidence that the balance and cross-regulation between the PI 3-kinase/Akt and Ras/Raf/MEK signaling cascades determine the temporal pattern of ERK1/2 phosphorylation and may thereby guide the phenotypic modulation of vascular smooth muscle cells.



Schematic diagram of the PDGF- and thrombin-mediated regulation of ERKs. PI 3-kinase-dependent sustained Akt activation following ligand binding of PDGF _-receptors inhibits Raf kinase activity and suppresses MEK and subsequent late phase ERK activation. The short-lived Akt activity following stimulation of a proteaseactivated receptor fails to inhibit Raf kinase activity. The Gβγ-induced activation of the Ras/Raf/MEK cascade results in a second-phase ERK phosphorylation required for the enhanced expression of contractile proteins. *PDGF* β*-R,* PGDF β-receptor; *PAR,* protease-activated receptor; *PI 3-kinases*$_{\alpha,\beta,\gamma}$ α, β-, and γ-subtypes of phosphatidylinositol-3-kinase; *p-Akt,* activated (S-473-phosphorylated) Akt; *p-ERK,* phosphorylated p42/p44-forms of extracellular signal regulated kinase



Effect of LY294002 on the phosphorylation pattern of Akt, Raf, MEK, and ERK in PDGF- and thrombin-stimulated cells.

11.2.3 Regulation of Raf-Akt Cross-talk

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Regulation of Raf-Akt Cross-talk

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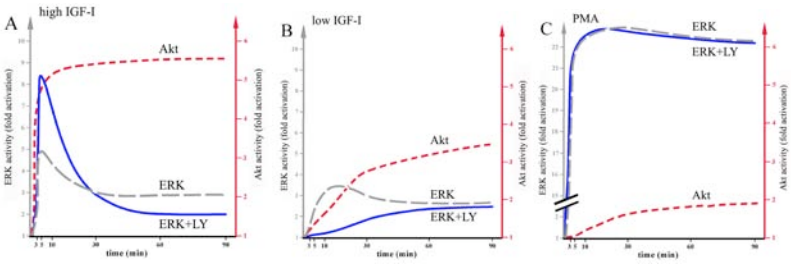
We have recently shown that the Ras-Raf-MEK-ERK and phosphatidylinositol 3-kinase (PI3K)-Akt signaling pathways can cross-talk in the human breast cancer cell line MCF-7. High Raf activity induces growth arrest and differentiation in these cells, whereas high PI3K/Akt activity correlates with cell survival and proliferation. Here we show that the Raf-Akt cross-talk is regulated in a concentration- and ligand-dependent manner. High doses of insulin-like growth factor I (IGF-I) activate Akt quickly and strongly enough to suppress Raf kinase activity via phosphorylation of Ser-259, whereas low doses of IGF-I do not trigger this cross-talk but are still mitogenic. Phorbol 12-myristate 13-acetate, a differentiation-inducing stimulus, potently activates the Ras-Raf-MEK-ERK pathway but only weakly activates PI3K/Akt and does not trigger the cross-talk. Thus, the herein analyzed parameters such as ligand type, concentration, and time course may contribute to the cellular response of either proliferation or differentiation. This is highly relevant to understanding cellular transformation and may be of use in areas like tissue engineering.

(6–11), which trigger biological responses via direct impact on gene expression.

Another important pathway that is triggered by IGF-I or insulin via phosphorylation of insulin receptor substrate, IRS-1, is the phosphatidylinositol 3-kinase (PI3K)-Akt pathway. PI3K is activated by binding of its p85 regulatory subunit to tyrosine-phosphorylated IRS-1. Activation of PI3K increases the amounts of membrane-localized phosphatidylinositol 3,4-bisphosphate and phosphatidylinositol 3,4,5-triphosphate. One of the crucial downstream targets of PI3K is the serine/threonine kinase Akt (12). Akt is recruited to the membrane by direct binding of its pleckstrin homology domain to the PI3K-produced phospholipids (13). Upstream kinases such as 3-phosphoinositide-dependent protein kinase 1 (PDK1) activate Akt by phosphorylation on Thr-308 (14) and Ser-473 (15).

Active Akt causes a variety of biological effects, including suppression of apoptosis by phosphorylation and inactivation of several targets along pro-apoptotic pathways such as the Bcl-2 family member BAD (16, 17) or caspase-9 (18). Moreover, it regulates glucose uptake by a largely uncharacterized mecha-

Fig. 1 Fig. 2



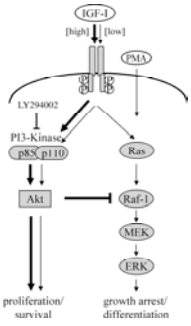


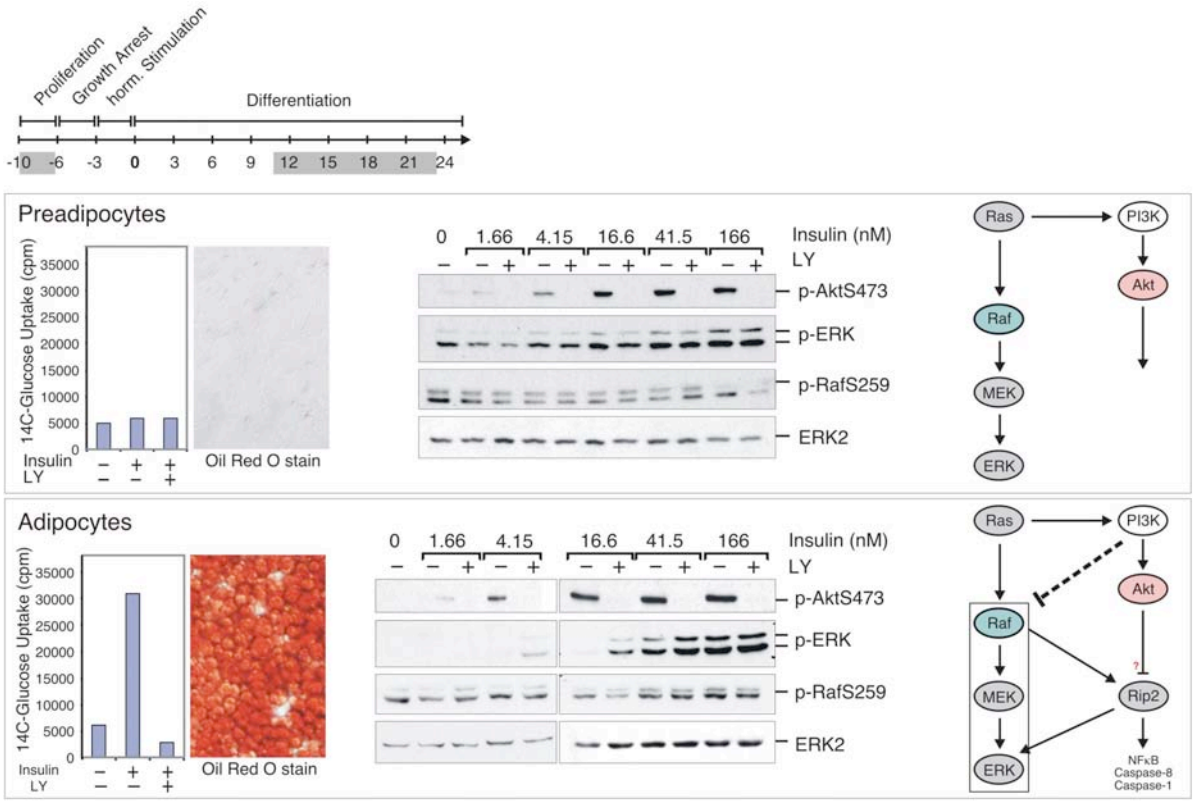
Fig. 1: Schematic summary of kinetics of Akt and ERK activity. (A) High proliferative conditions (high IGF-I concentrations) cause strong and persistent Akt activity in MCF-7 cells, whereas activation of ERK peaks after about 10 minutes and then decreases. Inhibition of PI3K and Akt by LY294002 leads to a stronger ERK activity at early time points but later activation of ERK is inhibited. (B) Slowly proliferating cells (low IGF-I concentrations) have reduced Akt and ERK activity, and inhibition of PI3K/Akt by LY294002 diminishes the ERK kinase activation. (C) In growth-arrested MCF-7 cells (PMA), Akt activity is weak, whereas activation of ERK is strong and persistent. The LY294002 inhibitor does not interfere with ERK activation.

Fig. 2: A model for the Akt-Raf interaction in MCF-7 cells. Phosphorylation of Raf by Akt leads to cross talk and inhibition of the Ras-Raf-MEK-ERK cascade and induction of proliferation in the presence of high IGF-I concentration (thick arrows). At low IGF-I concentration (thin arrows) Akt does not influence Raf-Kinase activitiy, a weak activation of the PI3K-Akt pathway leads to maintainance of proliferation/survival. Inhibition of PI3K by the inhibitor LY294002 and stimulation of the Ras-Raf-MEK-ERK pathway under these conditions and by the PMA are shown.

11.2.4 PI3K-dependent inhibition of ERK activation in 3T3-L1 adipocytes

Magnus Bosse, Jochen Heinrich and Karin Moelling
(PhD Thesis September 2004)

Negative regulation of the Raf/MEK/ERK pathway by PI3K/AKT via phosphorylation of S259 on Raf by AKT has been demonstrated in various cellular systems such as the human breast cancer cell line MCF-7. Since differentiation of adipocytes is known to be inhibited by Raf kinase activity we hypothesized that AKT may negatively regulate Raf activity also in this differentiation system. Using an inducible retroviral vector expressing the constitutively active Raf kinase domain we confirmed that Raf kinase activity inhibits adipogenesis of murine 3T3-L1 fibroblasts. Insulin, the physiological stimulus of adipocytes, activates the PI3K/AKT pathway and the Raf/MEK/ERK pathway. The regulation of the Raf/MEK/ERK pathway in insulin-stimulated cells was studied using the PI3K inhibitor LY294002. Upon insulin-stimulation a negative regulation was demonstrated for 3T3-L1 adipocytes, whereas in proliferating preadipocytes the ERK activity was unaffected. However, the inhibition was observed only with lower insulin concentrations within the physiological range or during the first 5 minutes of stimulation with high insulin concentration. We could not detect a PI3K-dependent phosphorylation of S259 on Raf in 3T3-L1 adipocytes. Moreover, c-Raf and B-Raf are only weakly activated in 3T3-L1 adipocytes. ERK appears to be activated by additional kinases, which in turn can be negatively regulated by PI3K/ AKT. A physiological role of the inhibition of ERK activation may be the prevention of the ERK-mediated negative phosphorylation of the Insulin receptor substrate 1 (IRS1). The receptor interacting kinase Rip-2, recently described as a Raf-dependent kinase of ERK, may contribute to the activation of ERK. Since Rip-2 is a putative AKT substrate, the ERK activation would be negatively regulated by PI3K. Furthermore, a negative regulation of pro-apoptotic Rip-2 by AKT would favor differention versus apoptosis.

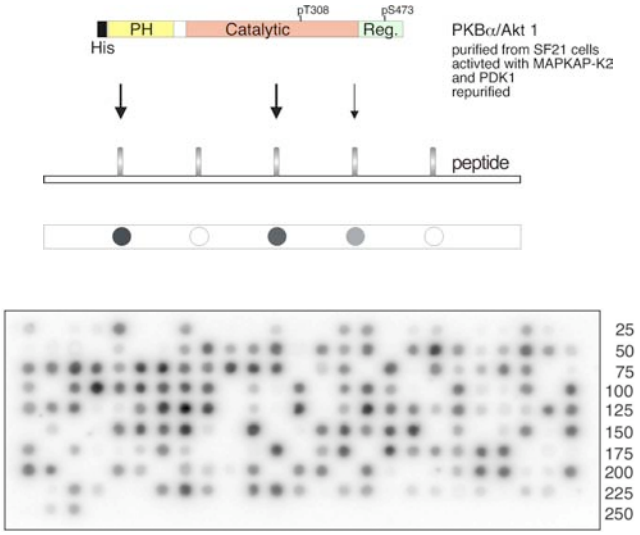


PI3K negatively regulates ERK activity in differentiated 3T3-L1 adipocytes, but not in proliferating preadipocytes. A contribution of Rip-2, recently described as a Raf-dependent kinase of ERK, is investigated.

11.2.5 AKT and Ras/Raf Signaling

Jochen Heinrich, Elisabeth Ortner and Karin Moelling

AKT negatively regulates RAF in particular cellular situations. On the one hand we want to further investigate the reason, why this cross-talk depends on the state of the cell. On the other hand AKT has many substrates. Therefore, we are interested to identify new AKT substrates involved in Ras/Raf signaling. In a peptide screen for putative AKT substrates harboring an AKT phosphorylation site that overlaps a 14-3-3 binding site we identified several peptides which were phosphorylated by purified AKT. Among these were signaling molecules related to Raf. One is the Ras effector RalGEF, the other was the MAP kinase kinase kinase 6. Additionally, the Ras effector AF6 exhibits putative AKT phosphorylation sites. Therefore we decided to study the role of AKT in the Ras/Raf signaling network.



Identification of AKT substrates

Peptides from putative substrates synthesized on cellulose membranes (collaboration with Ronald Franck, GBF, Braunschweig) were phosphorylated with purified AKT (left). Some substrates are listed.

	Protein	relative phosphorylation normalized to Bad
1	RAL GUANINE NUCLEOTIDE DISSOCIATION STIMULATOR-LIKE 2	295.0
2	RETINOBLASTOMA BINDING PROTEIN 1 (RBBP-1)	284.0
3	PROBABLE G PROTEIN-COUPLED RECEPTOR GPR15	218.5
4	EXTRACELLULAR CALCIUM-SENSING RECEPTOR PRECURSOR (CASR)	219.4
5	PROTEIN-TYROSINE PHOSPHATASE-LIKE N PRECURSOR (R-PTP-N)	206.3
6	KINESIN-LIKE PROTEIN KIF4	208.0
7	TUMOR SUPPRESSOR PROTEIN DCC PRECURSOR	197.0
8	VOLTAGE-DEPENDENT CALCIUM CHANNEL GAMMA-3 SUBUNIT	186.9
9	C-C CHEMOKINE RECEPTOR TYPE 6 (C-C CKR-6)	175.2
10	HIGH AFFINITY INTERLEUKIN-8 RECEPTOR A (IL-8R A) (CXCR-1)	145.6
11	MITOGEN-ACTIVATED PROTEIN KINASE KINASE KINASE 6	140.4
12	Raf1-259:	135.2*
13	PROTEIN KINASE C-LIKE 1	115.5
14	NEUROPEPTIDE Y RECEPTOR TYPE 5 (NPY5-R) (NPY-Y5 RECEPTOR)	109.8
15	GLYCOGEN [STARCH] SYNTHASE, BRAIN	106.2*
16	BAD PROTEIN	100.0*

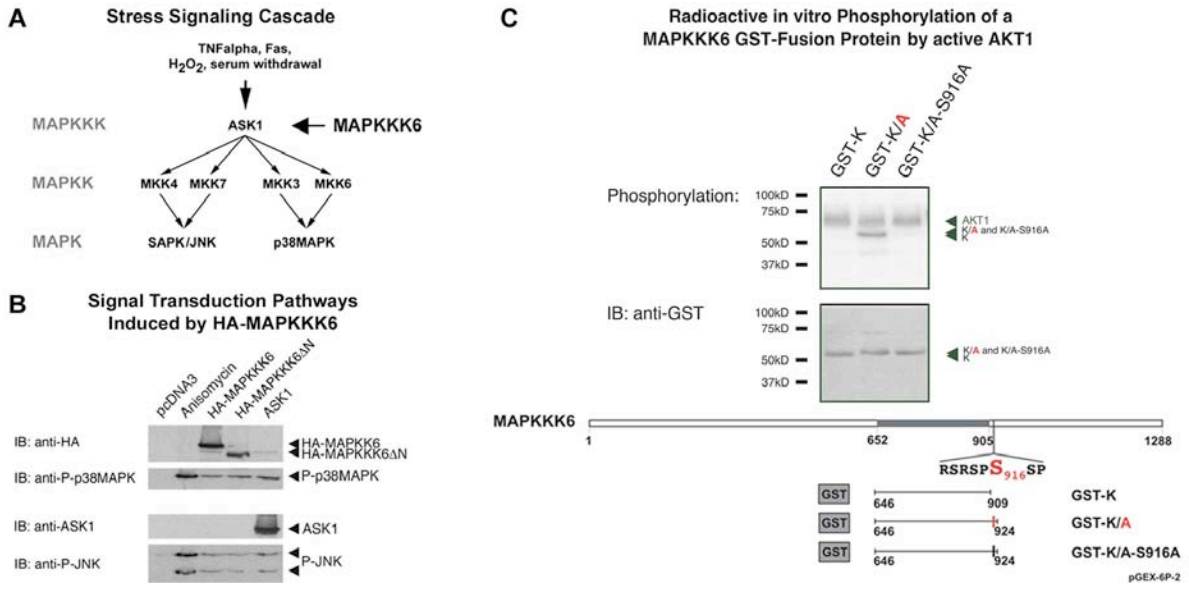
* known Akt substrates

11.2.6 Investigation of MAPKKK6 as a putative AKT substrate

Elisabeth Ortner, Jochen Heinrich, and Karin Moelling (Thesis ongoing)

AKT kinase is integrated in signaling pathways of distinct cellular fates such as proliferation, differentiation and apoptosis. Crosstalk between AKT and Raf, which induces 14-3-3 binding to Raf, is involved in the differentiation of C2C12 myoblasts to myotubes and in proliferation of MCF-7 breast cancer cells (1,2,3). In order to find new AKT substrates similar to Raf, a library of peptides with putative AKT phosphorylation and overlapping 14-3-3 binding sites was phosphorylated in vitro. Among others a peptide with a sequence of MAPKKK6 (mitogen activated protein kinase kinase kinase 6) was phosphorylated. This sequence is localized C-terminal to the kinase domain. MAPKKK6 has previously been described as homologous binding partner of MAPKKK5/ASK1 (apoptosis signal regulating kinase 1), and is mainly expressed in muscle tissues in contrast to the ubiquitously found ASK1. ASK1, an inducer of apoptosis, is negatively regulated by AKT phosphorylation at the N-terminal Ser83 which is no 14-3-3 binding site. In the present study an interaction of MAPKKK6 and AKT could be shown by coimmunoprecipitation of overexpressed Myc-MAPKKK6 with overexpressed HA-AKT in HEK-293 cells. Moreover, a GST-fusion protein consisting of the kinase domain plus the putative AKT recognition sequence was phosphorylated by active AKT1 in vitro. Upon serum deprivation overexpressed HA-MAPKKK6 was able to induce the phosphorylation of the stress signaling kinases JNK and p38MAPK in HEK-293 cells. The role of MAPKKK6 in the induction of apoptosis and a putative regulation of this cellular process by AKT are currently investigated.

(1): Zimmermann and Moelling, Science 286, 1741-4 (1999)
(2): Rommel et.al., Science 286, 1738-41 (1999)
(3): Moelling et.al., J Biol Chem, 277(34), 31099-106 (2002)



A: Schemativ overview of the stress signaling cascade. **B:** Overexpressed HA-MAPKKK6 induces the activation of the stress signaling kinases JNK and p38MAPK in serum derprived HEK-293 cells.**C:** Recombinant active AKT1 phosphorylates a GST-MAPKKK6 fusion protein at Ser916 in a radioactive in vitro phosphorylation assay.

11.2.7 Raf-1 inducible genes

ORIGINAL ARTICLE

Jochen Heinrich · Magnus Bosse · Holger Eickhoff
Wilfried Nietfeld · Richard Reinhardt · Hans Lehrach
Karin Moelling

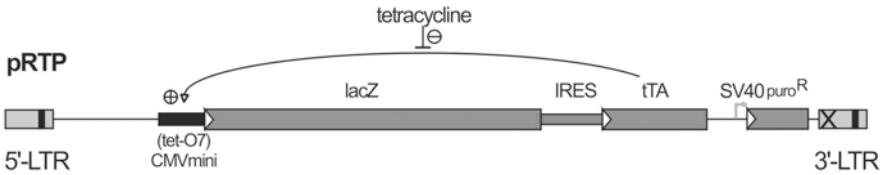
J Mol Med (2000) 78:380–388
DOI 10.1007/s001090000116

Induction of putative tumor-suppressing genes in Rat-1 fibroblasts by oncogenic Raf-1 as evidenced by robot-assisted complex hybridization

Received: 20 December 1999 / Accepted: 18 April 2000 / Published online: 11July 2000

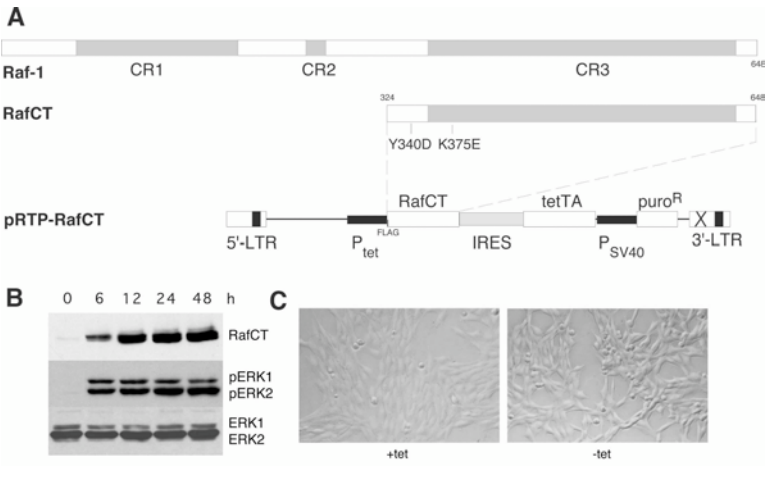
*in collaboration with the Max-Planck.Institut für Molekulare Genetik, Berlin

The growth factor receptor-dependent protein kinase Raf-1 is activated by GTP-bound Ras thereby activating the mitogen-activated protein kinase pathway. In order to study the role of Raf in transformation we transduced Rat-1 cells with a tetracycline-regulatable retroviral vector encoding the constitutively active oncogenic C-terminal fragment of the human Raf-1 protein. Using subtractive hybridisation of mRNAs from induced and non-induced cells and robot-assisted screening by complex hybridisation, Raf-induced genes with different characteristics of induction were investigated. Among the strongly induced genes were genes involved in carcinogenesis such as metalloproteinases 3, 10 and 13, cathepsin L, ornithine decarboxylase and putative tumor suppressing genes such as monocyte chemoattracting protein 1, interferon-induced protein 10, a recently identified 2'-5' oligoadenylate synthetase-like protein, and plasminogen activator inhibitor type 2. Other components of the plasminogen activator system were not induced. PAI-2 is a downregulator of the proteolytic cascade consisting of various metalloproteinases some of which are induced by RafCT. In conclusion RafCT induces factors, which act in a conflicting manner in respect of carcinogenesis, especially within the proteolytic system of the extracellular matrix.



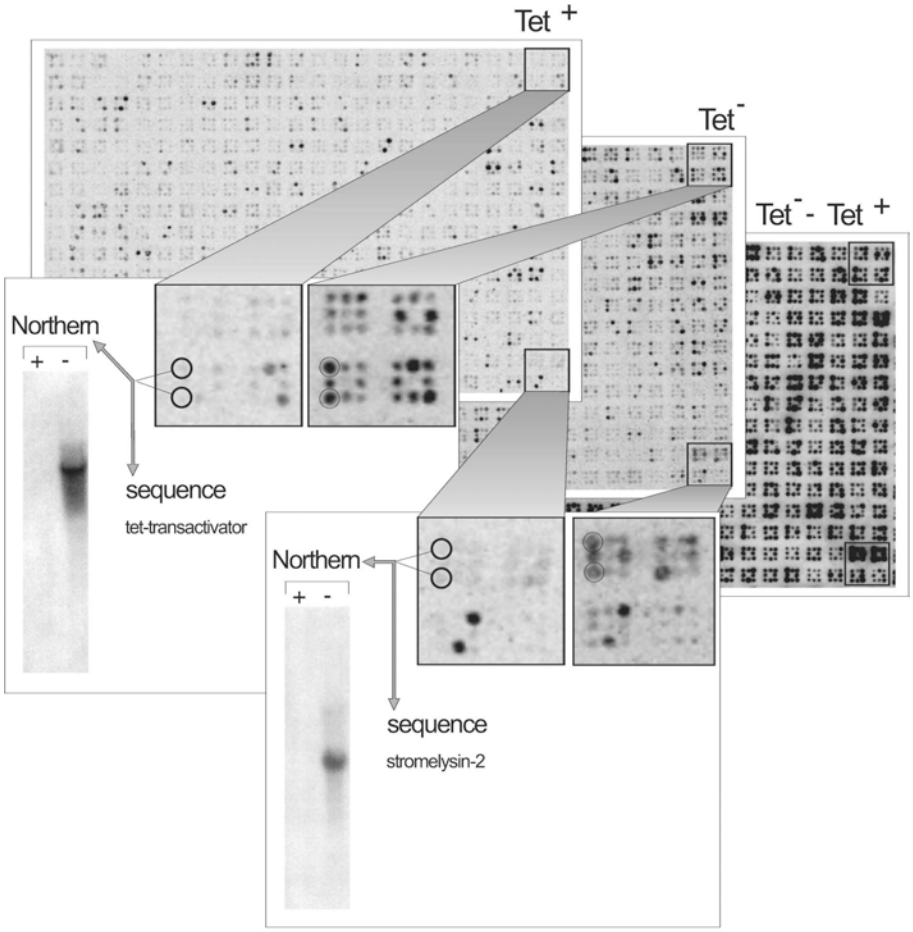
Tet-regulatable retroviral vector pRTP

Low promoter activity of the CMVmini promoter leads to transcription of an mRNA that is initiated upstream of the gene of interest (e.g. lacZ gene), progresses through the transactivator sequence (encoding a fusion protein of the tet-repressor and the transcriptional activation domain of herpes simplex virus VP16 protein) and ends in the R region of the retroviral 3'-LTR. Behind the lacZ gene an internal ribosomal entry site (IRES) has been inserted allowing the translation of the transactivator. The puromycin resistance gene is driven by the immediate early promoter of SV40. Gossen and Bujard have developed the tet system and have shown that tetracyclin prevents the transactivator(tTA) from binding to the operator (tet-O7). In the absence of tetracycline the transactivator binds to the tet operator thereby inducing increased protein expression. In addition, the vector has a defective 3'-LTR resulting in a defectve 5'-LTR after reverse transcription and provirus formation thereby allowing the (tetO7)CMVmini specific control of the gene of interest.



Inducible expression of RafCT

The C-terminal half of the c-raf-1 gene encoding the constitutively active C-terminal kinase domain (RafCT) was cloned in the tet-regulatable retroviral vector pRTP. Rat-1 cells transduced with pRTP-RafCT were cultured in the presence of tetracycline. The RafCT mutant was induced for the indicated time by tet-removal and RafCT expression and ERK phosphorylation was analyzed. After 24h in the absence of tet (–tet) Rat-1/RTP-RafCT cells are morphologically transformed, whereas non-induced cells (+tet) are not transformed.



Differential Hybridisation

To identify differentially expressed genes bacterial colonies resulting from a subtractive hybridsation for RafCT induced genes were spotted on nylon membranes to generate identical copies of gene fragments enriched for RafCT-inducible genes. The bacteria were lysed and the DNA was immobilized.The Filters were hybridised using labelled cDNA libraries from repressed (Tet^+) and induced (Tet^-) Rat-1/pRTP-RafCT cells as well as a cDNA library enriched for RafCT-induced genes (Tet^--Tet^+). The inserts of differentially expressed clones were used as probes for Northern analysis to confirm Raf-CT inducibility.

RafCT-induced genes

The fold induction was determined from northern blots.

Gene	Fold induction
Plasminogen activator inhibitor type 2 (PAI-2)	100.0
MMP-3 (Stromelysin-1)	66.0
MMP-10 (Stromelysin-2)	25.0
MMP-13 (Collagenase 3)	15.0
RafCT-IRES-tetTA (RafCT-tetTA)	13.0
p59 2'-5' oligoadenylate synthetase-like protein (p59OASL)	13.0
Interferon-induced protein 10 (IP-10)	9.0
Monocyte chemoattracting protein 1 (MCP-1)	4.4
Fos-related antigen 1 (Fra-1)	4.3
Cathepsin L (Cath L)	4.2
Ornithine decarboxylase (ODC)	3.9
FGF-regulated protein 1 (FR-1)	3.3
Matrix metalloproteinase (MMP-X)	3.1
PACAP response gene 1 (PRG1)	2.8
Tyrosine phosphatase IA-2a-like protein (IA-2a)	2.2
Phosphoglyceratemutase Type B (PGM)	1.9
mu EST	1.8
rat EST	1.7
Ribosomal protein L38	1.5
Elongation factor 1-β	1.5

11.2.8 The WD-FYVE protein ProF targets the kinases Akt and PKCζ/λ to vesicles

Thorsten Fritzius, Jochen Heinrich, *et al*., and Karin Moelling (submitted)

WD-repeat proteins offer a platform for protein-protein interactions by folding into a propeller-like binding platform. We identified the novel propeller-FYVE protein, ProF, consisting of seven WD-repeats and a FYVE domain for binding to intracellular membranes (Fig. 1). ProF preferentially interacts with the activated protein kinases Akt/PKB and PKCζ/λ upon hormonal stimulation. Using adipocytes as a model system to study ProF function, we show that the kinases Akt2 and PKCζ/λ translocate with ProF to the plasma membrane in response to insulin stimulation, parallel to the glucose transporter type 4, Glut4. Overexpression of ProF led to increased glucose uptake (Fig. 2), while knock-down of ProF by siRNA led to reduced glucose uptake and altered Glut4 membrane staining in stimulated adipocytes. Thus, ProF functions as a transporter of kinases involved in insulin-dependent glucose metabolism in adipocytes (Fig. 3). The protein may be involved in other inducible secretory systems.

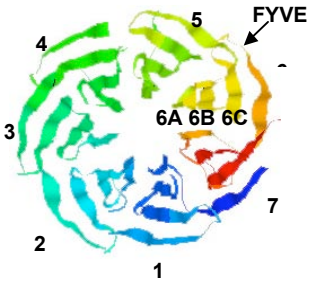


Fig 1: The protein ProF exhibits an unusual structure consisting of a FYVE motif for binding to vesicular phospholidips and seven WD40 repeats. Many WD repeats are known to serve as adaptor, by providing an platform for protein- protein interaction. Modeling approaches were performed for additional analysis of the overall protein structure. The calculated fold - generated by application of the 3D-PSSM program with known WD repeat proteins as structural template - shows a β-propeller characterized by seven blades arranged around a central axis. The FYVE domain, located between WD6 and WD7, is excluded from the modeling approach.

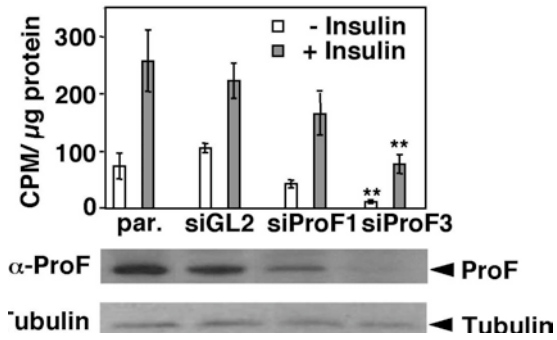


Fig. 2: Knockdown of ProF by siRNA decreases glucose uptake in adipocytes. 3T3-L1 cells were untransduced (par.), transduced with a retroviral expression vector for an unrelated siRNA (siGL2), or for two different siRNAs targeted against ProF mRNA (siProF1, siProF3). Serum-starved adipocytes were stimulated for 30 min without (open columns) or with 100 nM insulin (filled columns) before incubation with deoxyglucose for 10 min. Data are mean values ± standard deviations of 6 experiments. $^{**}P < 0.01$ (top panel). Different levels of ProF expression were analyzed by IB (top lane), total protein levels were standardized to tubulin (bottom lane).

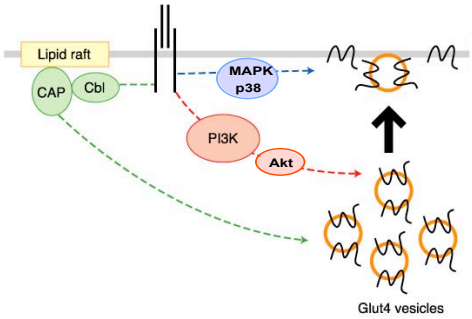


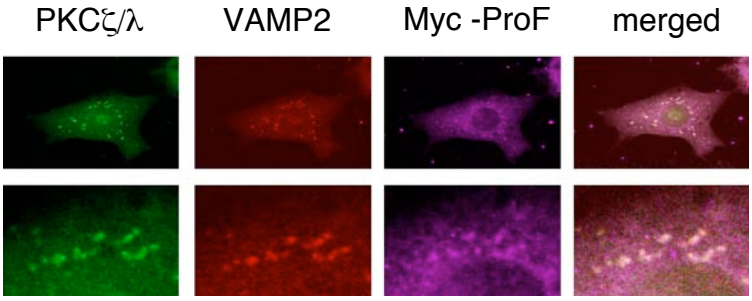
Fig. 3: Insulin regulation of glucose transport. Insulin may activate three independent signaling pathways, two of which trigger translocation of intracellular Glut4 vesicles and a third that controls "intrinsic" activity of the transporter at the plasma membrane.

[Adapted from K. V. Kandror, A long search for Glut4 activation. *Sci. STKE* **2003**, pe5 (2003)]

11.2.9 ProF, a WD-repeat protein, binds to PKCζ and VAMP2 for stimulation-dependent phosphorylation

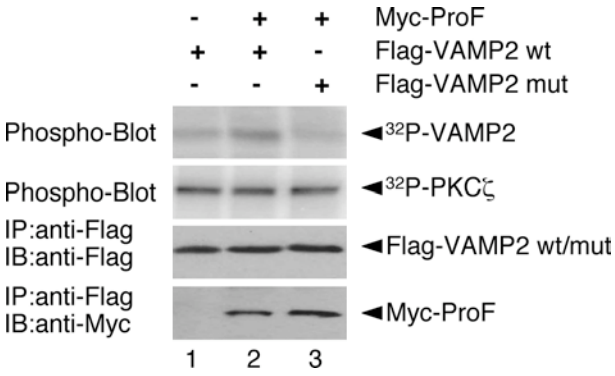
Thorsten Fritzius, et al., and Karin Moelling (submitted)

We have identified a protein, consisting of seven WD- repeats, presumed to form a β-propeller, and a FYVE domain, ProF, as a binding partner for the activated kinases Akt and PKCζ/λ. More recently, we found that vesicle associated membrane protein 2, VAMP2 is a further interaction partner of ProF. This interaction is demonstrated in mammalian cells, both with overexpressed and endogenous proteins. Several binding sites are involved in the binding of VAMP2 to ProF. ProF interacts with VAMP2 and PKCζ on vesicular structures. PKCζ, VAMP2, and ProF form a ternary complex, whereby ProF leads to increased binding of PKCζ to VAMP2. ProF recruits PKCζ to VAMP2 improving phosphorylation of VAMP2 *in vitro*. VAMP2 regulates docking and fusion of vesicles with cellular membranes in interacting with soluble N-ethylmaleimide-sensitive factor attachment receptor, SNARE, proteins at the plasma membrane. We propose that in 3T3-L1 adipocytes the ProF- mediated binding of PKCζ to VAMP2 and phosphorylation of VAMP2 plays a role in translocation of glucose transporter 4, GLUT4, to the plasma membrane. The ternary complex may also be involved in other secretory pathways.



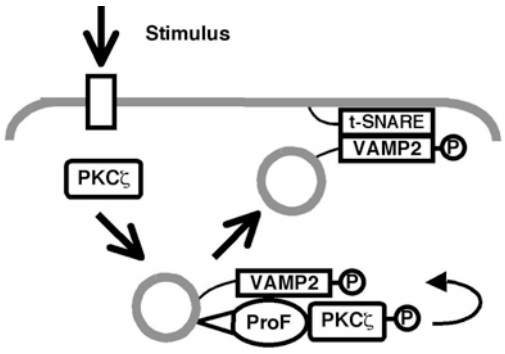
Colocaization of overexpressed Myc-ProF with endogenous PKCζ and VAMP2 on vesicular structures

To investigate the subcellular localization of ProF and its interaction partners PKCζ and VAMP2, confocal immunofluorescense analysis was performed with 3T3-L1 preadipocytes, stably transduced with a retroviral vector expressing Myc- tagged ProF. The cells were starved for 2 hours prior to staining for endogenous PKCζ/λ (green), endogenous VAMP2 (red), and Myc- ProF (blue). A partial colocalization on cytoplasmic punctuate structures (white) can be observed (merged and merged, zoomed).



ProF enhances *in vitro* phosphorylation of VAMP2 by PKCζ

Flag-epitope tagged VAMP2 wild type (Flag-VAMP2 wt) or serine to alanine mutant (Flag-VAMP2 mut) was overexpressed in the presence (lane 2 – 3) or absence (lane 1) of Myc-ProF in HEK 293T cells. Flag-VAMP2 was phosphorylated by addition of recombinant active PKCζ with ^{32}P-γ-ATP for 30 minutes. VAMP2 phosphorylation and PKCζ autophosphorylation was analyzed using a phosphorimager (1st and 2nd panel) and immunoprecipitation of proteins by immunoblotting against Flag- epitope (3rd panel) and Myc- epitope (4th panel). Thus, the presence of Myc-ProF improves *in vitro* phosphorylation of Flag-VAMP2.



Model of the general role of ProF in secretory systems

Stimulation of the cell induces a signal transduction cascade, which leads to activation of PKCζ, which is recruited to VAMP2-containing vesicles through the protein ProF. ProF enables the interaction between PKCζ and the v-SNARE VAMP2 leading to phosphorylation of VAMP2 by activated PKCζ. The vesicle then translocated to the plasma membrane, where the interaction of VAMP2 with its cognate t-SNARE induces incorporation of the vesicle into the plasma membrane.

11.3 PDZ-domains in signal transduction

Leitprojekt Molekulare Medizin: Proteinmodule von Signalkomplexen und deren Liganden als Ansatz für die molekulare Intervention (1998-2003)

Schneider S, Buchert M, Georgiev O, Catimel B, Halford M, Stacker SA, Baechi T, Moelling K, Hovens CM. Mutagenesis and selection of PDZ domains that bind new protein targets. **Nature Biotech. 17**, 170-175 (1999)

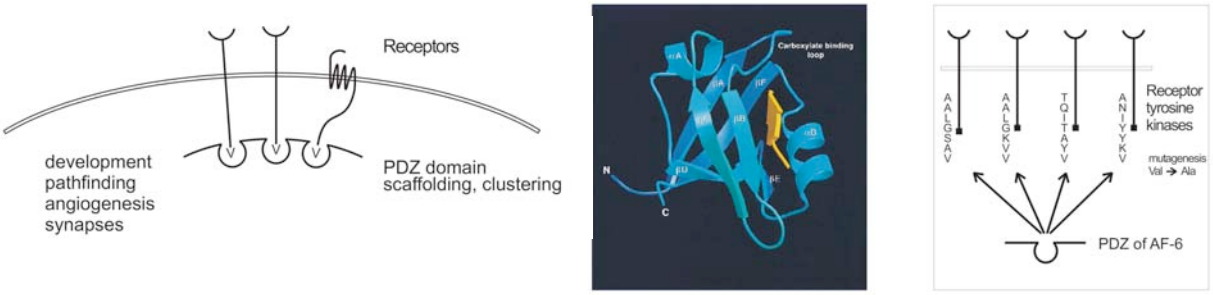


Figure: (left) PDZ domain containing proteins are scaffold proteins involved in different biological processes. One function also studied in the IMV is receptor clustering of Eph receptors by the PDZ protein AF-6 (Buchert M, Schneider S, Meskenaite V, Adams MT, Canaani E, Baechi T, Moelling K, Hovens CM. The junction-associated protein AF-6 interacts and clusters with specific Eph receptor tyrosine kinases at specialized sites of cell-cell contact in the brain. J Cell Biol. 1999 Jan 25;144(2):361-71). (middle) Structure of the PDZ3 domain of PDZ-95 (aa 309-393) (Doyle et al., 1996). (right) In vivo evolution of non-binder to binder (Schneider *et al.*, 1999).

11.3.1 Search for novel signaling molecules using the yeast two-hybrid system

Gerald Radziwill, Rüdiger Erdmann, Angelika Ress and Karin Moelling

To search for interaction partners of selected proteins we used a yeast two-hybrid system based on the yeast transactivator protein Gal4 (Fields and Song, 1989). So far, the following cDNA-libraries have been used: human B-cell library in pACT derived from Epstein-Barr-Virus transformed, peripheral lymphocytes (obtained from S. Elledge), human T-cell library in pGAD10 derived from a T-cell population of Jurkart cell line (CLONTECH Laboratories Inc.), human brain MATCHMAKER cDNA-libraries in pACT2 (CLONTECH Laboratories Inc.). Several novel interaction partners obtained with different bait proteins are now under further investigation (see table and following chapters).

Table: Protein-protein interactions identified by the yeast two hybrid system

bait-plasmid	interaction partner	interacting domain
ErbB2CT	ERBIN	PDZ
RykCT	AF-6	PDZ
EphrinBCT	Jab1	MPN
GABA-R1aCT	c6.1A	MPN
Rafwt	14-3-3	RSTS259TP
RafS259A	TIP60	?
MEK1	B-Raf	C-terminus
Akt1	AIP-1/AIP-2	WD repearts/ ?
CNK1	Clk1, FHOS, SH3GLB1	C-terminus

11.3.2 Mutagenesis and selection of PDZ domains that bind new protein targets

Stefan Schneider, Michael Buchert, Oleg Georgiev[1], Bruno Catimel[2], Michael Halford[2], Steven A. Stacker[2], Thomas Baechi[3], Karin Moelling*, and Christopher M. Hovens[5]

*Institut für Medizinische Virologie, [3]Elektronenmikroskopisches Zentrallabor, Universität Zürich, Gloriastr. 30/32, CH-8028 Zürich, Switzerland. [1]Institut für Molekularbiologie II, Universität Zürich, 8057 Zurich, Switzerland. [5]New address: Department of Surgery, [2]Ludwig Institute for Cancer Research, Royal Melbourne Hospital, Clinical Sciences Building, Parkville VIC. 3050 Australia. *Corresponding author (e-mail: moelling@immv.unizh.ch).*

Nature Biotech. **17**, 170-175 (1999)

PDZ domains are a recently characterized protein-recognition modules. In most cases, PDZ domains bind to the C-terminal end of target proteins and are thought thereby to link these target proteins into functional signaling networks. We report the isolation of artificial PDZ domains selected via a mutagenesis screen in vivo, each recognizing a different C-terminal peptide. We demonstrate that the PDZ domains isolated can bind selectively to their target peptides in vitro and in vivo. Two of the target peptides chosen are the C-terminal ends of two cellular transmembrane proteins with which no known PDZ domains have been reported to interact. By targeting these artificial PDZ domains to the nucleus, interacting target peptides were efficiently transported to the same subcellular localization. One of the isolated PDZ domains was tested and shown to be efficiently directed to the plasma membrane when cotransfected with the full-length transmembrane protein in mammalian cells. Thus, artificial PDZ domains can be engineered and used to target intracellular proteins to different subcellular compartments.

11.3.3 The Junction-associated protein AF-6 interacts and clusters with specific Eph receptor tyrosine kinases at specialized sites of cell-cell contact in the brain

Michael Buchert,* Stefan Schneider,* Virginia Meskenaite,§ Mark T. Adams,* Eli Canaani,¶ Thomas Baechi,‡ Karin Moelling,* and Christopher M. Hovens*

*Institut für Medizinische Virologie and ‡Elektronenmikroskopisches Zentrallabor, Universität Zürich; §Institut für Neuroinformatik, Universität Zürich/Eidgenössische Technische Hochschule, CH-8028 Zürich, Switzerland; and ¶Weizmann Institute for Science, Rehovot 76100, Israel

J Cell Biol. **144**, 361-71 (1999)

The AF-6/afadin protein, which contains a single PDZ domain, forms a peripheral component of cell membranes at specialized sites of cell-cell junctions. To identify potential receptor-binding targets of AF-6 we screened the PDZ domain of AF-6 against a range of COOH-terminal peptides selected from receptors having potential PDZ domain-binding termini. The PDZ domain of AF-6 interacts with a subset of members of the Eph subfamily of RTKs via its COOH terminus both in vitro and in vivo. Cotransfection of a green fluorescent protein-tagged AF-6 fusion protein with full-length Eph receptors into heterologous cells induces a clustering of the Eph receptors and AF-6 at sites of cell-cell contact. Immunohistochemical analysis in the adult rat brain reveals coclustering of AF-6 with Eph receptors at postsynaptic membrane sites of excitatory synapses in the hippocampus. Furthermore, AF-6 is a substrate for a subgroup of Eph receptors and phosphorylation of AF-6 is dependent on a functional kinase domain of the receptor. The physical interaction of endogenous AF-6 with Eph receptors is demonstrated by coimmunoprecipitation from whole rat brain lysates. AF-6 is a candidate for mediating the clustering of Eph receptors at postsynaptic specializations in the adult rat brain.

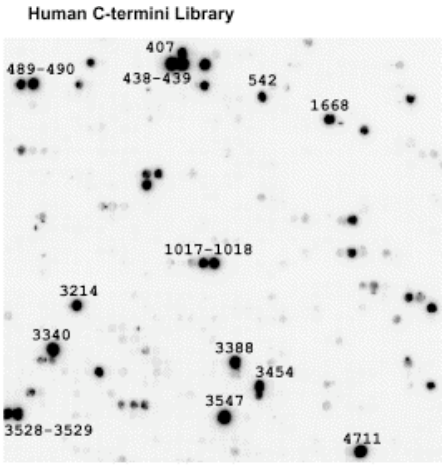
11.3.4 Proteinmodule von Signalkomplexen und deren Liganden als Ansatz für die molekulare Intervention (Leitprojekt Molekulare Medizin)

Gerald Radziwill, Jochen Heinrich and Karin Moelling together with Hartmut Oschkinat (FMP, Berlin)

Prisca Boisguerin, Rainer Leben, Bernhard Ay, Gerald Radziwill, Karin Moelling, Liying Dong, and Rudolf Volkmer-Engert. An Improved Method for the Synthesis of Cellulose Membrane-Bound Peptides with Free C Termini Is Useful for PDZ Domain Binding Studies. Chem. Biol. 11: 449-459(2004)

Wiedemann U, Boisguerin P, Leben R, Leitner D, Krause G, Moelling K, Volkmer-Engert R, Oschkinat H. Quantification of PDZ domain specificity, prediction of ligand affinity and rational design of super-binding peptides. J Mol Biol. 343, 703-18 (2004)

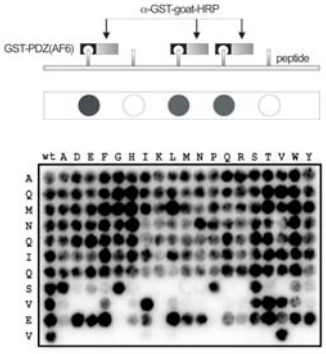
PDZ Domänen sind Adaptordomänen, die bei der Signalübertragung, bei der Strukturbildung des Zytoskeletts, bei Zell-Zell Kontakten u.a. eine Rolle spielen. Sie sind charakterisiert durch eine hochkonservierte hydrophobe Tasche mit den Aminosäuren GLGF. In diese Tasche greifen Liganden ein, wobei carboxyterminale Enden mit endständigem V,I,L bevorzugt werden. Diese Tasche ist für drug-targeting von Interesse. Die Wechselwirkung mit Liganden ist reguliert und spezifisch. Das Ziel dieses Projekts ist es, PDZ Domänen-haltige Proteine als potentiell krankheitsrelevante Targets zu untersuchen und Ansätze für die Behandlung von Krankheiten durch Hemmung PDZ-abhängiger Interaktionen zu finden.

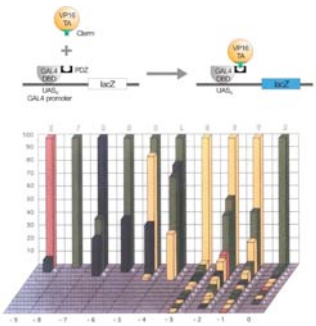


Identification of PDZ ligands: Inverted Peptide Arrays on Cellulose Membranes for Protein Binding: Extract from a library of 6223 C termini (11-mers) of human proteins from the SWISS-PROT database incubated with GST-labeled ERBIN PDZ domain (cysteine replaced by serine). The 30 strongest binders are listed in the table below.

Spot	Sequence	Accession No.	Kd [µM]	Protein Description
102	FSTALYGESDL	P12814		alpha-actinin 1, cytoskeletal isoform
103	FSSALYGESDL	P35609		alpha-actinin 2, skeletal muscle isoform
116	LQDEKVKESYV	O95477		ATP-binding cassette, sub-family A, member 1
131	KRNTLYFSTDV	Q12979		active breakpoint cluster region-related protein
220	SSLREMETFVS	P50052		type-2 angiotensin II receptor (AT2)
336	RHSGSYLVTSV	P25054		adenomatous polyposis coli protein (APC protein)
407	DAKPQPVDSWV	O00192	8,0 ± 3,1	armadillo repeat protein deleted in velo-cardio-facial syndrome [26]
438	GSPLHSLETSL	P20020	24,5 ± 5,0	plasma membrane calcium-transporting ATPase, isoform 1
439	GSPIHSLETSL	Q01814	54.2 ± 4.8	plasma membrane calcium-transporting ATPase, brain isoform 2
441	DSSLQSLETSV	P23634		plasma membrane calcium-transporting ATPase, isoform 4
489	NVDFPPKESSL	P27037		activin receptor type II precursor
490	NVDLPPKESSI	Q13705		activin receptor type IIb precursor
505	GCLREWCDAFL	O75531		barrier-to-autointegration factor
542	KRQSILFSTEV	P11274	36.0 ± 5.2	breakpoint cluster region protein
788	FKLLDQMETPL	P08311		cathepsin G precursor
1017	CSNAKAVETDV	P22459		voltage-gated potassium channel protein KV1.4
1018	LCLDTSRETDL	P22460	79.4 ± 2.8	voltage-gated potassium channel protein KV1.5
1027	TVRPGVKESLV	P35499	82.7 ± 1.3	sodium channel protein, skeletal muscle alpha-subunit
1168	KINLSQKETSI	P53618		coatomer beta subunit
1253	NSRPHTNETSL	P23508		colorectal mutant cancer protein
1284	APQWVPVSWVY	O14936		peripheral plasma membrane protein CASK
1300	SNQLAWFDTDL	P35222	53.1 ± 0.5	beta-catenin
1351	IGTMFLRETSL	P33402	115.5± 9.2	guanylate cyclase soluble, alpha-2 chain
1668	SPQWVPVSWVY	Q00013		55 kDA erythrocyte membrane protein (p55)
1958	KQASSQSWVPG	O43524		forkhead protein O3A
3214	SQKVAVYSTCL	P07942		laminin beta-1 chain precursor
3340	TLDSQIQETSI	P27816		microtubule-associated protein 4
3388	VKRMRMDAWVT	Q02078		myocyte-specific enhancer factor 2A
3454	LGARVSKETPL	P53778		mitogen-activated protein kinase 12
3528	EPQWVPVSWVY	Q14168		MAGUK p55 subfamily member 2
3529	DTHWVPVSWVR	Q13368		maguk p55 subfamily member 3
3547	PSTLTIFETAL	O15439		multidrug resistance-associated protein 4
3807	WRRISSLESEV	Q14957		glutamate [NMDA] receptor subunit epsilon 3 precursor
3848	LLSDMYKSSDI	Q9Y466		orphan nuclear receptor NR2E1
3882	GLIAGEKETHL	P48065		sodium- and chloride-dependent betaine transporter
4711	QLNGTFESQVQ	P25800		rhombotin-1
5027	DGGRDQQETNL	Q92911		sodium/iodide cotransporter
5028	SSTCILQETSL	Q9Y289		sodium-dependent multivitamin transporter
5840	QSFLQTETSVI	P41587		vasoactive intestinal polypeptide receptor 2 precursor
5956	MNDPAWDETNL	Q14155		hypothetical protein KIAA0142

Specificity of the PDZ domain: The specificity of the PDZ domain is analysed using peptide profiling (above) and the yeast twohybrid system (below).





11.3.5 The Bcr kinase downregulates Ras signaling by phosphorylating AF-6 and binding to its PDZ domain

Gerald Radziwill, Rüdiger A. Erdmann, Ulrike Margelisch, and Karin Mölling *Mol. and Cell. Biol.* **23**, 4663-4672 (2003)

The protein kinase Bcr is a negative regulator of cell proliferation and oncogenic transformation. We identified Bcr as a ligand for the PDZ domain of the cell junction- and Ras interacting protein AF-6. The Bcr kinase phosphorylates AF-6, which subsequently allows efficient binding of Bcr to AF-6 showing that the Bcr kinase is a regulator of the PDZ domain-ligand interaction. Bcr and AF-6 co-localize in epithelial cells at the plasma membrane. In addition, Bcr, AF-6 and Ras form a trimeric complex. Bcr increases the affinity of AF-6 to Ras and a mutant of AF-6 that lacks a specific phosphorylation site for Bcr shows a reduced binding to Ras. Bcr wild type but not Bcr mutants defective in binding to AF-6 interferes with the Ras-dependent stimulation of the Raf/MEK/ERK pathway. Since AF-6 binds to Bcr via its PDZ domain and to Ras via its Ras-binding domain, we propose that AF-6 functions as a scaffold-like protein that links Bcr and Ras to cellular junctions. We suggest that this trimeric complex is involved in down-regulation of Ras-mediated signaling at sites of cell-cell contact to maintain cells in a non-proliferating state.

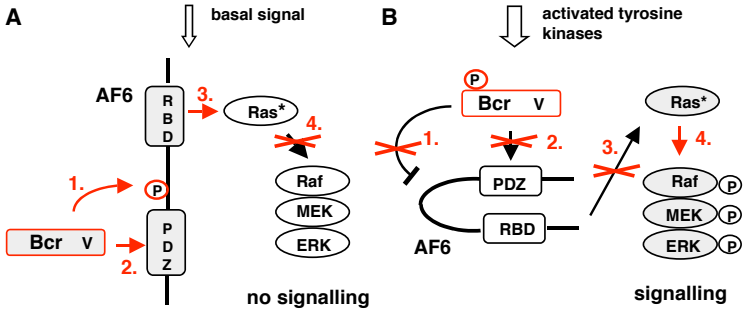


Figure: Possible model depicting the effect of Bcr on Ras-dependent stimulation of ERK via AF-6. (A) In quiescent cells the constitutively active Bcr phosphorylates AF-6 (step 1) which leads to the interaction of the PDZ domain of AF-6 with the PDZ binding motif of Bcr (step 2). This interaction increases the affinity of AF-6 for Ras (step 3) and prevents binding of Raf to Ras (step 4). Under these conditions the protein kinase cascade composed of Raf, MEK, and ERK is not activated. (B) Phosphorylation of Bcr on tyrosine residues inactivates its protein kinase activity. Therefore, Bcr cannot phosphorylate (step 1) and cannot bind to AF-6 (step 2). Thus, AF-6 does not compete with Raf for Ras (step 3) and does not interfere with the Ras-dependent activation of the protein kinase cascade (step 4). P represents phosphorylation of proteins.

Based on the identification of Thr893 of AF-6 as Bcr phosphorylation site we postulated a consensus phosphorylation site for Bcr; E/D-X-S/T-X-E/D-E/D. Protein data base search revealed several putative substrates, among them 14-3-3τ, the only known substrate for Bcr apart from AF-6. The Ser/Thr kinase Pak1 was identified as substrate of Bcr *in vitro*.

postulated Bcr phosphorylation site:	protein	peptide sequence	Bcr substrate
E/D-X-S/T-X-E/D-E/D	AF-6(T893)	VVAVAENT893 ADELARS	+
		VVAVAENV893 ADELARS	–
	AF-6(S1533)	LQSKPDRS1533AEESDRL	?
	14-3-3 τ	TEQGAELS 37 NEERNLL	+
		TEQGAELV 37 NEERNLL	–
	14-3-3 ε	AGMDVELT 38 VEERNLL	–
	Pak1	YMSFTDKS149 AEDYNSS	+
		YMSFTDKV149 AEDYNSS	–
	MKK4/JNKK1	PEQHWDFT 98 AEDLKDL	–
	CHK2	VFVFFDLT205 VDDQSVY	?

Figure: Selected peptides containing the postulted Bcr phosphorylation site were tested for phosphorylation by Bcr *in vitro*.

11.3.6 CNK1 is a scaffold protein that regulates Src-mediated Raf-1 activation

Algirdas Ziogas, Karin Moelling, and Gerald Radziwill

***J. Biol. Chem.* 280**, 24205-24211 (2005)

Raf-1 is a regulator of cellular proliferation, differentiation and apoptosis. Activation of the Raf-1 kinase activity is tightly regulated and involves targeting to the membrane by Ras and phosphorylation by various kinases including the tyrosine kinase Src. Here we demonstrate that the connector enhancer of Ksr1, CNK1, mediates Src-dependent tyrosine phosphorylation and activation of Raf-1. CNK1 binds preactivated Raf-1 and activated Src and forms a trimeric complex. CNK1 regulates the activation of Raf-1 by Src in a concentration-dependent manner typical for a scaffold protein. Down-regulation of endogenously expressed CNK1 by small inhibitory RNA interferes with Src-dependent activation of ERK. Thus, CNK1 allows a cross-talk between Src and Raf-1 and is essential for full activation of Raf-1.

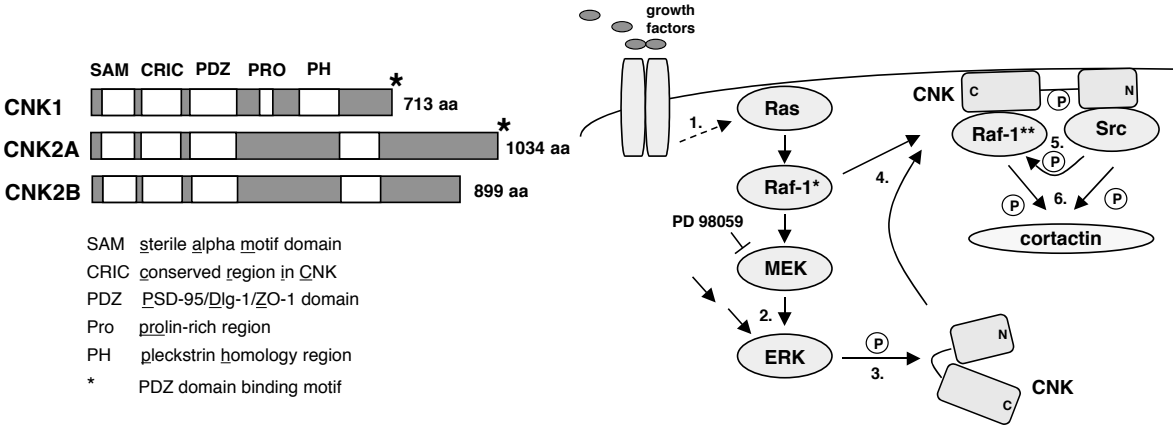


Figure: (left) Domain structure of human CNK proteins. (right) Proposed model for the function of CNK1 in Raf signaling. Upon activation of cells Ras stimulates the Raf-1/MEK/ERK kinase cascade. This results in plasma-membrane recruitment and preactivation of Raf-1 (Raf-1*) as well as ERK-dependent phosphorylation and plasma-membrane recruitment of CNK1 (steps 1-4). Preactivated Raf-1 interacts with CNK1 and can be tyrosine phosphorylated and fully activated (Raf-1**) by CNK-1 bound Src (step 5). Cortactin is a substrate for Src and Raf-dependent sigalling (step 6).

To further characterize the function of CNK1 we performed a yeast two-hybrid screen to identify CNK1-interacing proteins. Several novel CNK1 partners, including kinases, adaptor proteins and proteins involved in cell trafficking, were identified and will be further investigated.

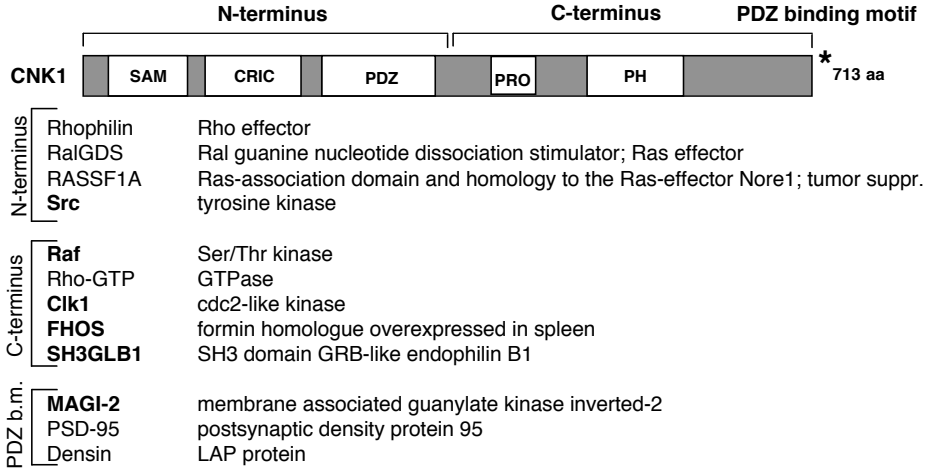


Figure: Interaction partners for CNK1identified here (bold) and known from the literature. Apart from MAGI-2 the PDZ binding partners are only described for CNK2A.

11.3.7 The scaffold protein CNK1 interacts with the angiotensin II type 2 receptor

Rafael D. Fritz* and Gerald Radziwill
(* diploma work, *Biochem. Biophys. Res. Commun.,* in press)

The multidomain protein CNK functions as a scaffold that connects upstream activators and downstream targets of Ras- and Rho-dependent signaling pathways and may allow cross-talks between theses pathways. CNK mediates proliferative as well as antiproliferative responses including differentiation and apoptosis depending on the cellular system.

The AT_2 receptor is an atypical G protein-coupled receptor that negatively cross-talks with receptor tyrosine kinases to inhibit cell proliferation or to promote neuronal differentiation and apoptosis. Antiproliferative effects of the AT_2 receptor are mainly exerted by the activation of protein phosphatases coupled to the inhibition of the Raf/MEK/ERK signal cascade.

In this study we describe a physical interaction of CNK1 with the AT_2 receptor in an overexpression system as well as in mouse heart. We identified two binding sites located in the N-terminal portion of CNK1, namely the sterile alpha motif (SAM) and the conserved region in CNK (CRIC) domain. Exchange of a conserved leucine residue in the CRIC domain increased the binding affinity of the CNK1 protein to the AT_2 receptor. An insertion of a negatively charged amino acid stretch into the linker region between the N- and the C-terminal part of CNK1 strengthens the interaction between CNK1 and the AT_2 receptor in a Ras-regulated manner. This indicates that the activation state of a cell modulates the binding between CNK1 and the AT_2 receptor.

What could be the functional significance of the CNK1/AT_2 receptor interaction? First, CNK proteins as well as the AT_2 receptor act in differentiation of neuronal cells mediated by the Rap1/B-Raf/MEK/ERK pathway (Figure, part A). Second, CNK and the AT_2 receptor can modulate ERK activity not only in neuronal cells but also in other cell systems. The AT_2 receptor can induce anti-proliferative signaling by stimulating the protein phosphatases SHP-1, MKP-1and PP2A (Figure, part B). A complex formed of the AT_2 receptor, CNK and Raf may facilitate dephosphorylation of Raf by PP2A and thereby downregulates mitogenic effects. Third, CNK1 and the AT_2 receptor both can activate caspase-3 to drive cells into apoptosis (Figure, part C). Therefore CNK1 may be linked with the AT_2 receptor-induced apoptosis. Thus, CNK may allow the interaction of the AT_2 receptor with its downstream targets and thereby mediate anti-proliferative effects including growth inhibition, differentiation and apoptosis.

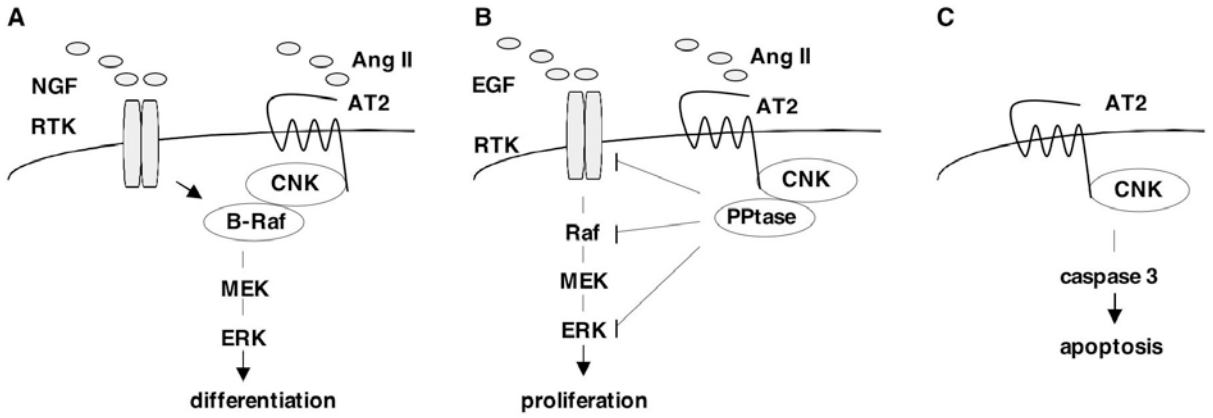


Figure: Putative functions of CNK in AT2-dependent signal pathways. NGF: nerve growth factor; EGF: epidermal growth factor; Ang II: angiotensin II; RTK: receptor tyrosine kinase; PPtase: protein phosphatase. For details see text.

11.3.8 Negative Regulation of c-Src by its PDZ Domain-Binding Motif

Gerald Radziwill, Andreas Weiss, Jochen Heinrich, Mihaela Lorger, Martin Baumgartner, Koji Owada and Karin Moelling

Overexpression or increased activity of the proto-oncogene c-Src is frequently detected in human cancers. Here we report that the very C-term of c-Src displays a functional PDZ domain-binding motif which is conserved between the three major SFK members Src, Yes and Fyn. Via this motif, c-Src is able to specifically interact with PDZ domain proteins. Disruption of the c-Src PDZ domain-binding motif by mutating the hydrophobic Leucin 535 to Alanine impaires the c-Src – PDZ protein interaction. The c-Src/L535A mutant exhibits an increased kinase activity and promiscuity, leading to a higher phosphorylation of c-Src substrates. Furthermore, Src/Yes/Fyn triple knock out cells inducibly expressing the c-Src/L535A mutant show an increased cellular transformation, e.g. elevated formation of soft agar colonies and foci, migration, and detachment, compared to c-Src/WT expressing cells. Hence, we suggest a negative modulation and a spatial restriction of c-Src activity mediated by its C-terminal interaction with PDZ domain proteins

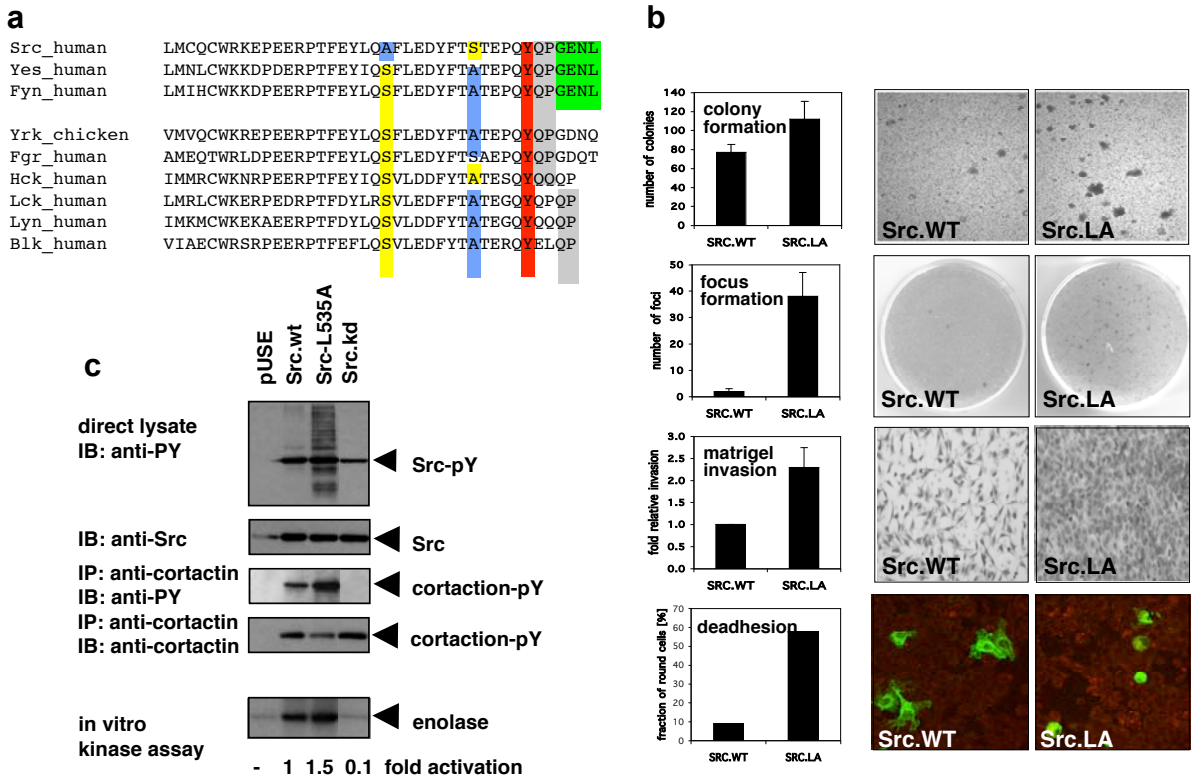


Figure: (A) C-terminal sequence alignment of Src-family kinases. Green: PDZ domain-binding motif. (B) Analysis of Src/Yes/Fyn knock out fibroblasts inducibly expressing c-Src/WT or c-Src/L535A for cellular transformation. (C) Analysis of HEK 293 cells transiently transfected with c-Src/WT or c-Src/L535A. Upper panel: cell lysates were analyzed with anti-pY antibodies. Middle panel: immunoprecipitation of the c-Src substrate cortactin and analysis of its phosphorylation state with anti-pY antibodies. Lower panel: c-Src kinase assay with enolase as substrate.

To further study the role of PDZ binding motif-mediated interactions of c-Src we performed a PDZ Array screen to identify c-Src-interacting PDZ domain proteins. We could identify two PDZ proteins interacting with c-Src in a PDZ-dependent manner. The biological function of these interactions will be further investigated.

11.3.9 Bcr is a negative regulator of the Wnt signaling pathway

Angelika Ress and Karin Moelling, *EMBO Rep*. **6**, 1095-1100 (2005)

The Wnt signaling pathway can activate transcription of genes such as *c-myc* via β-catenin. Here we describe the protein breakpoint cluster region, Bcr, as negative regulator of this pathway. Bcr can form a complex with β-catenin and negatively regulate expression of c-Myc. Knockdown of Bcr by siRNA relieves the block and activates expression of c-Myc. Expression of Bcr in the colon carcinoma cell line HCT116, which has a high level of endogenous β-catenin, leads to reduced c-Myc expression. The negative effect is exerted by the amino-terminus of Bcr, which does not harbor the kinase domain. Bcr-Abl, the oncogene expressed in chronic myelogenous leukemia, CML, does not bind to β-catenin. It phosphorylates Bcr within the first exon sequence on tyrosines, which abrogates the binding of Bcr to β-catenin. The inhibitor of the Bcr-Abl tyrosine kinase, STI-571 or Gleevec, a drug against CML, reverses this effect. Our data contribute to the understanding of Bcr as tumor suppressor in the Wnt signaling pathway as well as in CML.

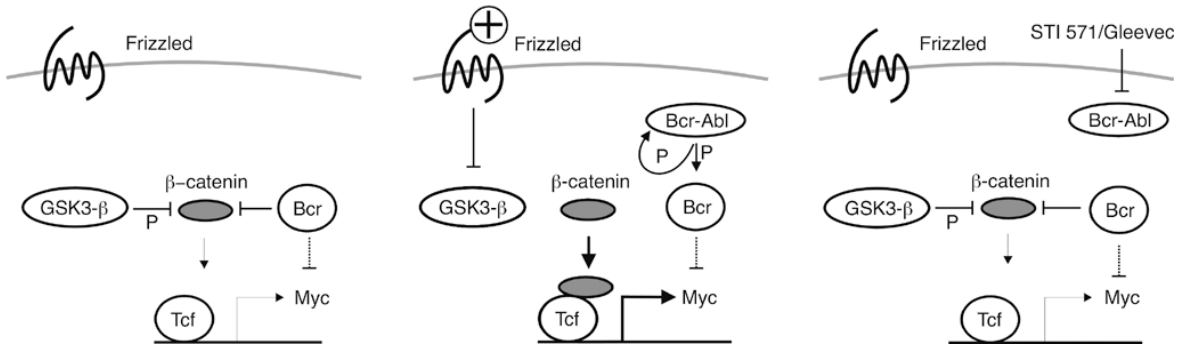


Figure: Model on Bcr effects on β-catenin. Negative regulation is indicated by bars, positive by arrows. Dotted lines indicate downregulation of c-Myc protein levels by Bcr (Mahon et al, 2003). Unstimulated cell (left), Bcr-Abl expressing tumor cell (middle), STI-571 effect (right). Thick arrows indicate strong activation of c-Myc.

11.3.10 Bcr interferes with β-catenin-Tcf1 interaction

Angelika Ress and Karin Moelling

The β-catenin/Tcf complex is a downstream effector of the Wnt signaling pathway. It is a transcription complex, which activates gene expression and contributes to proliferation and tumor progression. Tcf1 in complex with β-catenin is able to activate β-catenin dependent gene expression. We demonstrate that ectopically expressed Bcr is able to bind the transcription factor Tcf1 to interrupt the transcriptionally active β-catenin/Tcf1 complex. Phosphorylation of Bcr by tyrosine kinase $pp60^{src}$ can lead to dissociation of β-catenin/Tcf1 complex. Thus Bcr can have an inhibitory effect on Tcf1-dependent gene transcription, which is abrogated by phosphorylation of Bcr by the tyrosine kinase $pp60^{src}$. Thus, two independent mechanism may regulates β-catenin/Tcf-mediated transcription via Bcr, binding to β-catenin as previously shown and to Tcf1 as shown here.

11.3.11 Regulation of epithelial wound closure and cell-cell adhesion via AF6 interaction with actin cytoskeleton

M. Lorger and K. Mölling (submitted)

AF6 is a junctional protein involved in cell junction formation during mice embryogenesis and its *Drosophila* analogue, canoe, in the epithelial sheet migration during embryonic dorsal closure and possibly in cleft lip syndrome in humans. Three AF6 isoforms have been postulated **(Fig. 1 A)**, but only isoform 1 (AF6i1) was analyzed so far. Here we cloned the AF6 isoform 3 (AF6i3), which differ to AF6i1 by the presence of C-terminal F-actin binding site. AF6i3 knock-down in epithelial cells **(Fig. 1 B)**, which express only this isoform, accelerated wound closure **(Fig. 1 Ca)**, due to reduced cell-cell adhesion **(Fig. 1 Cc)** and increased migratory directionality **(Fig. 1 Cb)**, as revealed by time-lapse analysis. Concomitantly, de novo cell junction formation was impaired due to delayed recruitment of junctional proteins **(Fig. 1 D)**. AF6i1, lacking the F-actin binding site, failed to restore the wild type phenotype **(Fig. 1 E and F)**. Since AF6i3 knock-down did not alter cell junctions in static epithelial monolayers, our data identify AF6i3 and its interaction with the F-actin as a crucial component for the stabilization of cell-cell adhesion specifically during dynamic processes.

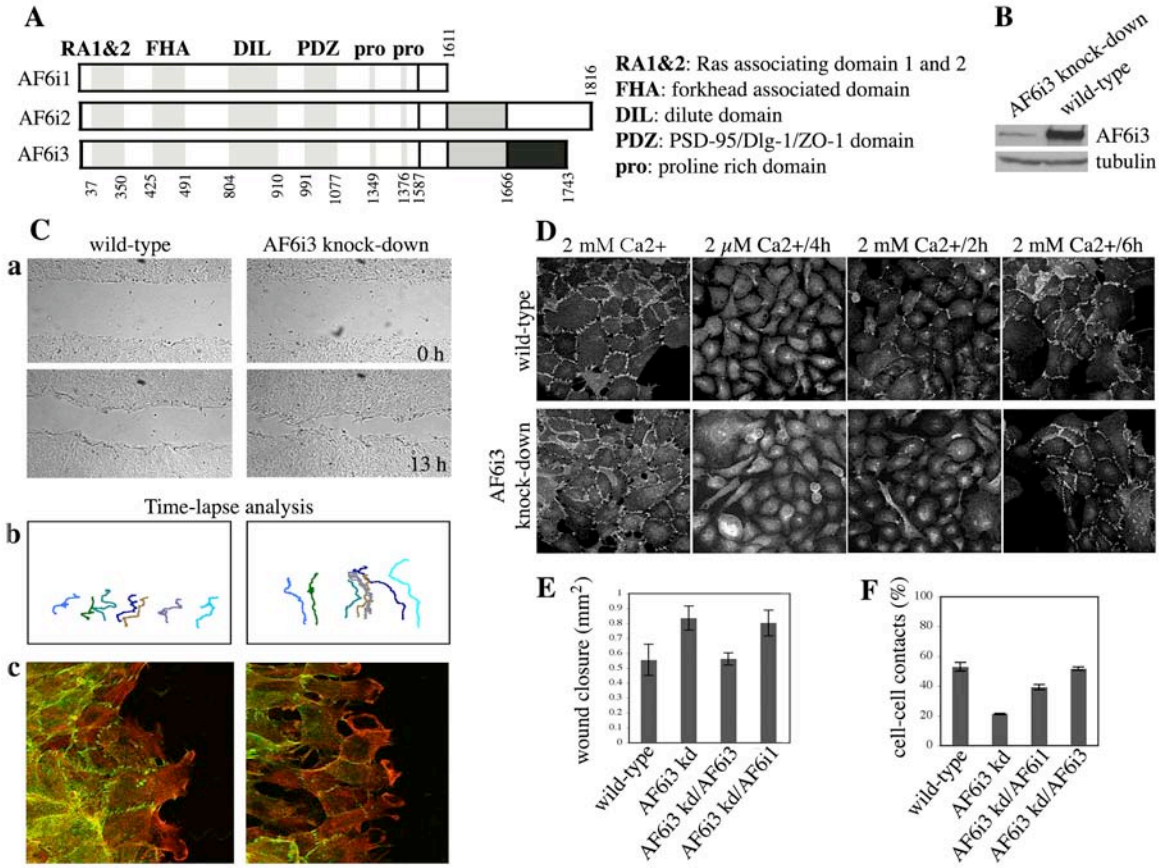


Fig.1. A. Domain structure of AF6 isoforms. **B.** Reduction of AF6 protein expression in MCF10A cells by shRNA. **C. (a)** Wound healing assay: confluent monolayar of MCF10A cells was scratched with a pipette (0h). Cell migration was initiated by adding 10 ng/mL EGF to the culture. The migration of the cells into the wound 13 h later is depicted. **(b)** Trajectories of MCF10A cells during wound closure, tracked for 10 h. **(c)** Wound margin 10 h after migration. Green signal: E-cadherin. Red signal: F-actin. **D.** Ca2+ switch assay: MCF10A cell monolayers (2 mM Ca2+) were incubated in DMEM/F12 medium with 5 mM EGTA for 4 h (2 mM Ca2+), which resulted in a dissociation of cell-cell contacts. The reformation of cell junctions 2 and 6 h after the switch to a higher calcium concentration (2 mM Ca2+/2h and 6h) was recorded by staining of the cells with anti-β-catenin antibody. **E.** Grapfic representation of wound closure 13 h after wound introduction for AF6i3 knock-down cells reexpressing the AF6i3 or AF6i1 protein. **F.** Grapfic representation of the Ca2+ switch assay with AF6i3 knock-down cells reexpressing the AF6i3 or AF6i1 protein. Percentage of newly formed cell-cell contacts 2 h after switching to 2 mM Ca2+ concentration is depicted.

11.4 The Antiviral Function of the Interferon System

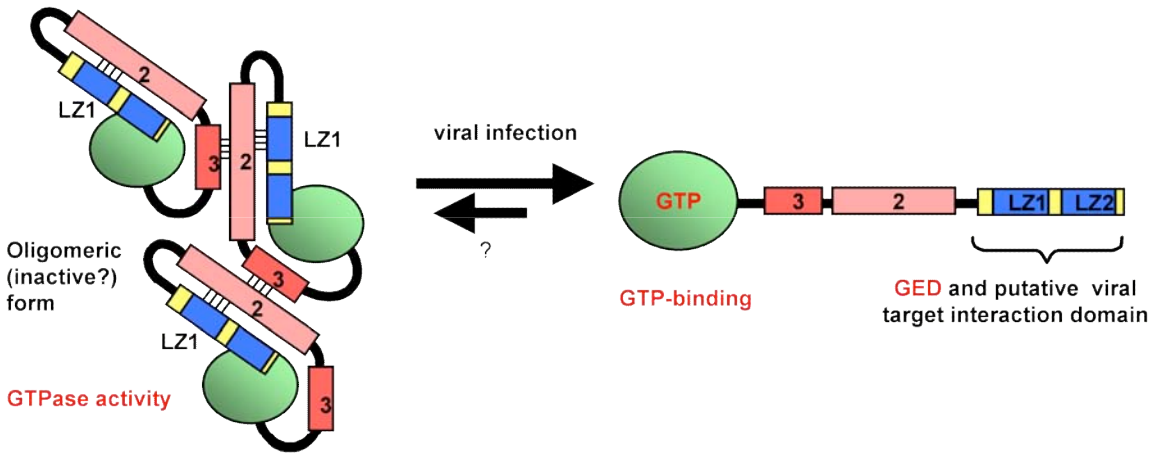
11.4.1 The Antiviral Function of the Interferon-Induced Mx Proteins

PD Dr. Jovan Pavlovic together with Eva Mantei, Stefan Deuber, Nathalie Constantin and semester students

The overall goal of this project is to elucidate the molecular mechanism of action of Mx proteins. This is achieved by: (a) assessing the antiviral spectrum of Mx proteins in cell culture and in vivo, (b) defining the functional domains of Mx proteins, (c) characterizing the viral target of Mx action and (d) identifying auxiliary factors necessary for Mx activity.

The GTPAse and antiviral activity of Mx proteins is controled by a carboxyterminal GTPase effector domain

The interferon-α/β inducible Mx proteins belong to the functionally diverse dynamin family of large GTPases. Characteristic features are a highly conserved GTP-binding region, an intrinsic GTPase activity and their capacity to form oligomers. The human MxA protein exerts antiviral activity by inhibiting the replication of certain RNA viruses in a GTP-dependent fashion. We have recently demonstrated that backfolding of the C-terminal end of human MxA protein onto a more proximal part of the molecule is a prerequisite for oligomerization. The backfolding of MxA is stabilized by an amphipathic helix LZ1. Prevention of backfolding by mutation of LZ1 at the amino acid position 612 (L612K) leads to the loss of GTPase activity and capacity to oligomerize, but the antiviral activity is retained. We show that similarly to dynamin, the intrinsic GTPase activity of Mx proteins is mediated by a GTPase effector domain (GED),. Based on these results we propose a model where Mx proteins are synthesized in response to interferon-α/β to form a pre-activated oligomeric structure. In the presence of viral components, MxA may then convert in a GTP-dependent manner to a monomeric, activated form. Once this step is completed MxA no longer requires GTP for its antiviral activity.



The GTPase Effector Domain (GED) and the proposed function of MxA

Intramolecular Backfolding of the Carboxyl-terminal End of MxA Protein is a Prerequisite for its Oligomerization, *Journal of Biological Chemistry,* 274, 32071-32078 (1999).

Human MxA Protein Protects Mice Lacking a Functional Alpha/Beta Interferon System against La Crosse Virus and Other Lethal Viral Infections, *Journal of Virology,* 73, 6984-6991 (1999).

11.4.2 Interferon Sensitivity of Semliki Forest Virus

Stefan Deuber and Jovan Pavlovic (Thesis, 2005)

Type I Interferons (IFNs) are secreted cytokines that represent an important component of the innate immune system. The induction of many genes by IFN leads to the generation of the so-called antiviral state in the cell. During evolution, viruses have developed various mechanisms to counteract the antiviral activity of the IFN system. Cells that lack a functional IFN system become very sensitive to normally harmless viruses demonstrating the importance of the IFN system in antiviral protection.

We are investigating two closely related Semliki forest virus (SFV) strains with different sensitivities to IFN. The virulent V45 strain is lethal for wild type (wt) mice whereas infection of mice with the avirulent V42 strain remains asymptomatic. Upon IFN treatment *in vitro*, MEFs derived from wt mice are completely protected from the avirulent V42 strain while IFN-stimulated cells are killed by the virulent V45 strain. We were interested in defining the viral sequence determining the IFN sensitivity of SFV.

We have sequenced both virus strains and constructed recombinant cDNA clones derived from both strains. Several differences were detected in the coding region of the non-structural (ns) and structural proteins (sPs) but also in the non-translated region (NTR). Even though more genomic RNA is synthesized by the avirulent strain V42 (figure 1), less nsPs are translated from this template (figure 2). Differences found in the 5' NTR may affect the translation efficiency of the incoming viral RNA. The nsPs have been described to interfere with the cellular translation machinery. A reduced translation rate of nsPs by the avirulent strain V42 may therefore decrease the translational host shut-off. A potent block of host protein expression early during infection appears to be important for the virus to interfere with IFN-induced proteins exhibiting antiviral activity and thereby determining virulence.

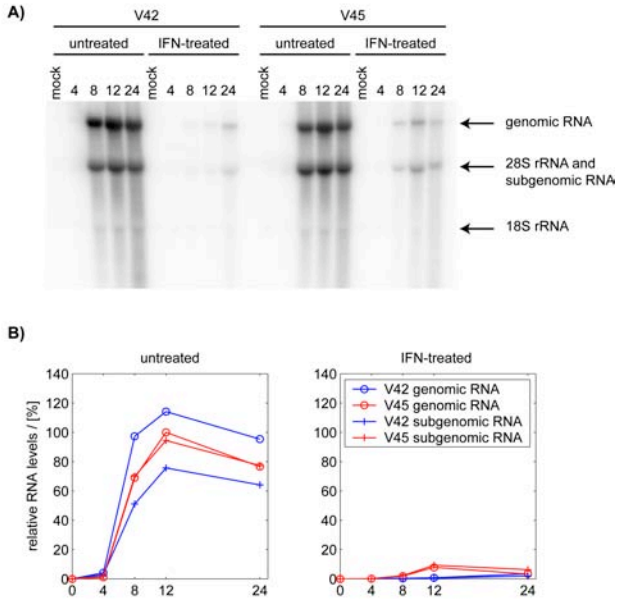


Figure 1: The avirulent SFV strain V42 synthesizes more genomic RNA than the virulent V45 strain. Temporal accumulation of genomic and subgenomic RNA was analyzed (A) and densiometrically quantified (B).

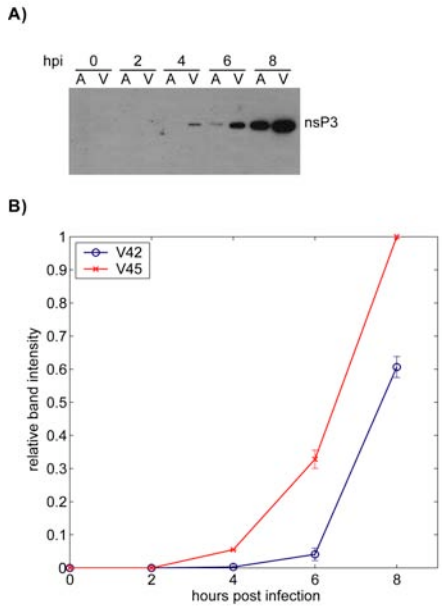


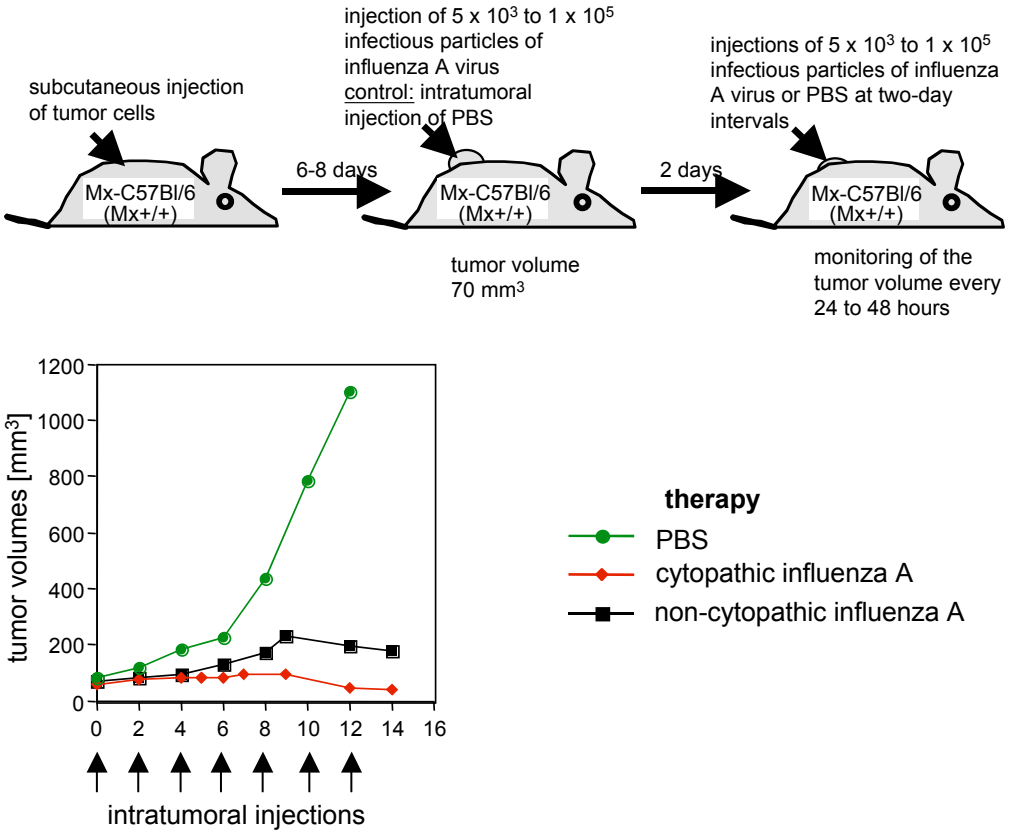
Figure 2: The avirulent SFV strain V42 produces less nonstructural proteins than the virulent V45 strain. nsP3 expression was detected at several timepoints after infection (A) and quantified (B).

11.5 Gene Therapy and HIV

11.5.1 Selective lysis of tumor cells with a deficiency in the interferon type I system with influenza A virus in vivo

Jovan Pavlovic, Jan Schultz and Karin Moelling (*Cancer Research,* under modification)

Tumor cells can acquire defects in the interferon type I system making them highly sensitive to the cytopathic effect of viruses in cell culture. To examine whether it is possible to selectively destroy tumors with influenza A viruses without affecting surrounding tissue, we used the mouse melanoma B16 tumor model. B16 cells are highly sensitive to infection with influenza virus due to a mutation in the interferon responsive gene Mx1. Treatment of established subcutaneous B16-tumors with multiple injections strongly reduced tumor growth irrespective of whether a highly cytopathic or non-cytopathic virus was used. Virus replication was essential for the oncolytic activity, since UV inactivation of Influenza virus completely abrogated its anti-tumoral effect. Analysis of lysed tumor cells revealed that virus treatment led to the induction of IFN-α, TNF-α, and TGF-β1, which are known to contribute to tumor cell death. The oncolytic activity of inluenza A virus was confined to the injected tumors, no systemic spreading of the virus was observed. Efficient reduction of tumor growth was also observed in mice vaccinated with the virus prior to tumor therapy, indicating that pre-existing immunity against the virus had no effect. A similar therapeutic strategy can be envisaged for human tumors like lung cancers, melanomas, and cutaneous lymphomas many of which can loose the interferon type I response thereby becoming highly susceptible to the cytopathic effects of viruses.



Tumor therapy with influenza A virus. Mx-C57BL/6 mice were injected subcutaneously with 5 x 10^5 tumor cells. As soon as the tumors reached a size between 20-50 mm^3, 1 x 10^5 infectious particles of influenza A virus were injected directly into the tumor. Injection of influenza A virus was repeated at least 5 times. Tumor volumes were monitored every 24 to 48 hours. Control mice were mock-infected with the same volume of PBS.

11.5.2 Silencing of HIV-Replication by an Oligonucleotides directed against the polypurine Tract by a Mechanism designed as siDNA

Karin Moelling et al.

Reverse Transcription of retroviral RNA into double-stranded DNA is catalyzed by the Reverse Transcriptase (RT). A highly conserved polypurine tract, PPT, which is found twice - in the *nef* gene adjacent to U3 and in the *pol* gene - in HIV-1 viral RNA, serves as primer for plus strand DNA synthesis. The PPT and seven additional purine nucleotides constitute a suitable target for triple-helix formation. An oligodeoxynucleotide (TFO A) was designed to form a triple-helix to block replication of HIV-1 in acutely infected T cells (1). *In vitro* TFO A has been shown to inhibit the RT and RNaseH activities (1). In cell culture experiments TFO A is an efficient inhibitor of retroviral replication leading to a strong and long-lasting reduction of p24 synthesis and inhibition of syncytia formation. The inhibitory effect was much superior to GEM91 - an antisense construct analyzed in clinical trials. Furthermore, acute infection with patient-derived viral isolates were inhibited for more than 14 days (2). A PCR analysis performed on HIV infected and TFO A treated cells indicated that DNA provirus formation inside the cell is inhibited (2). The molecular mechanism of TFO A on HIV replication is not yet known. Prediction of the potential secondary structure of the triplex-forming oligodeoxynucleotide suggests that an imperfect duplex structure may form. For further evaluation the effect of TFO A on HIV isolates harboring drug-resistant RT mutants were analyzed (3). The three isolates used exhibited resistance against azidothymidine, dideoxyinosine, deoxythiacytidine and nevirapine. Since replication of all the three mutant strains tested and the wild type isolate were completely inhibited by TFO A treatment, we conclude that TFO A interferes with functions of the replicative cycle distinct from polymerization by RT. The antiretroviral activity of TFO A in HIV replication may result from multiple synergistic effects targeting different replicative functions of the virus such as the RT, RNaseH-mediated initiation of second strand, and/or the integration step catalyzed by the integrase. Furthermore, a mutational analysis of TFO A in HIV-A infected cells was performed in order to reveal the mechanism.

Recently we were able to demonstrate that the TFO A does not inhibit HIV replication by triple-helix formation (4,5) but by activation of the retroviral RT/RNase H to prematurely cleave the viral RNA before its transcription into cDNA has been completed. The TFO A does not inhibit the RT/RNase H but activates it and is renamed as oligodeoxynucleotide ODN A. The ODN A mimicks a local cDNA at the PPT, which activates cleavage by the RT/RNase H and thereby cuts the viral RNA leading to HIV suicide. The ODN A antisense strand binds to the RNA, the passenger strand is essential for the phenomenon, it is sequence specific, its length is important. Its function may be stabilization of the antisense-strand. This phenomenon of RNA silencing is reminiscent of siRNA and therefore designated here as siDNA. The RT/RNase H is structurally related to the active enzyme component of the siRNA cleavage enzyme, the PAZ/PIWI domains of Ago2, which is part of RISC.

(1) Volkmann, S., Jendis J., Frauendorf, A., and Moelling, K. (1995) *Nucleic Acid Res* 23, 1204-1212.

(2) Jendis, J., Strack, B., Volkmann, S., and Moelling, K. (1996) *AIDS Res Hum Retroviruses* 12, 1161-1168.

(3) Jendis, J., Strack, B., and Moelling, K. (1998) *AIDS Res Hum Retroviruses* 14(11), 999-1005.

(4) Raetzel, N., Diploma Thesis, 2004.

(5) Quast, A., Diploma Thesis 2005.

11.5.3 Inhibition of HIV-1 Replication by TFO A

AIDS RESEARCH AND HUMAN RETROVIRUSES
Volume 14, Number 11, 1998, pp. 999–1005
Mary Ann Liebert, Inc.

Inhibition of Replication of Drug-Resistant HIV Type 1 Isolates by Polypurine Tract-Specific Oligodeoxynucleotide TFO A

JÖRG JENDIS, BETTINA STRACK, and KARIN MOELLING

ABSTRACT

A 54-base-long oligodeoxynucleotide (ODN) termed *triple helix-forming oligonucleotide A* (*TFO A*), designed against the 3′-polypurine tract (PPT) of the human immunodeficiency virus type 1 (HIV-1), exhibits long-term efficacy in antiretroviral treatment. Viral replication of strains propagated in this laboratory as well as primary patient isolates are inhibited by TFO A, whereas ODNs with a randomized sequence but identical base composition show no effect. TFO A inhibits proviral DNA synthesis. To learn more about the molecular mechanism of function of TFO A, three HIV-1 isolates whose reverse transcriptase (RT) exhibits resistance against RT inhibitors were analyzed. They exhibit resistance against azidothymidine, dideoxyinosine, deoxythiacytidine, and the nonnucleoside inhibitor nevirapine. HIV-1 replication in TFO A-treated T cell cultures was assessed by monitoring p24 viral core antigen production and syncytium formation. No p24 antigen or syncytia were detected for up to 30 days when cells that had been infected with wild-type virus received TFO A. Similarly, replication of all three mutant HIV-1 strains was completely inhibited by TFO A treatment during the whole duration of the culturing period. No viral breakthrough was detectable. These results indicate that TFO A interferes with functions of the replicative cycle distinct from polymerization by the RT.

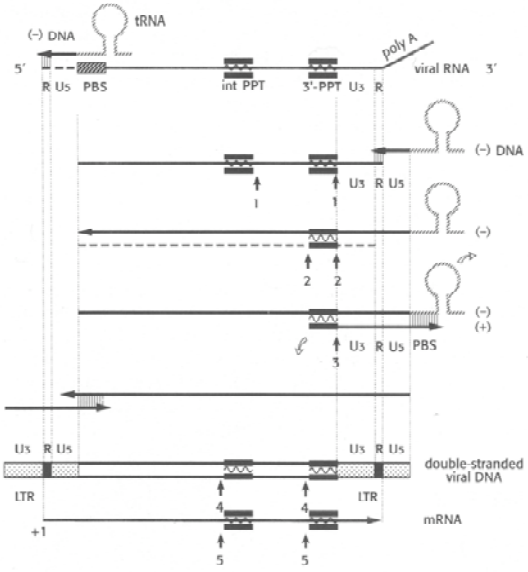


Figure 1. Model of the RT-catalyzed reverse transcription and the possible target-sites for triple-helix formation. The retroviral RNA genome contains two identical polypurine tracts (PPT) indicated by black bars. These polypurine sequences are potential target-sites for triple-helix formation which might interfere with reverse transcription at various steps. Reverse transcription of a poly(A) containing retroviral RNA genome initiates close to the 5′-end of the viral RNA at a tRNA primer which binds to a primer binding site (PBS). DNA synthesis continues at the 3′-end, whereby the two redundant regions R are involved. The reaction product of this minus-strand DNA synthesis is an RNA•DNA hybrid. The RNA moiety of this hybrid is hydrolyzed by the RNase H activity of the RT except for the PPT sequences which serve as primers for the plus-strand DNA synthesis. U_5 and U_3 indicate unique regions at the 5′- and 3′-ends, respectively. They become duplicated during double-stranded DNA formation and form the long terminal repeats, LTRs. Arrows and numbers point to events which might be inhibited by triple-helix formation: [1] inhibition of RNA-directed DNA synthesis; [2] inhibition of RNase H cleavage at the PPT which prevents the release of the primer for plus-strand DNA synthesis; [3] inhibition of initiation of plus-strand DNA synthesis; [4] inhibition of transcription of the double-stranded DNA provirus; [5] translation of the mRNA.

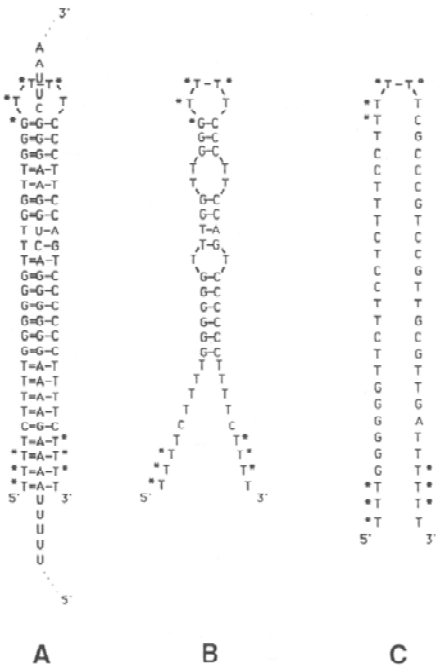
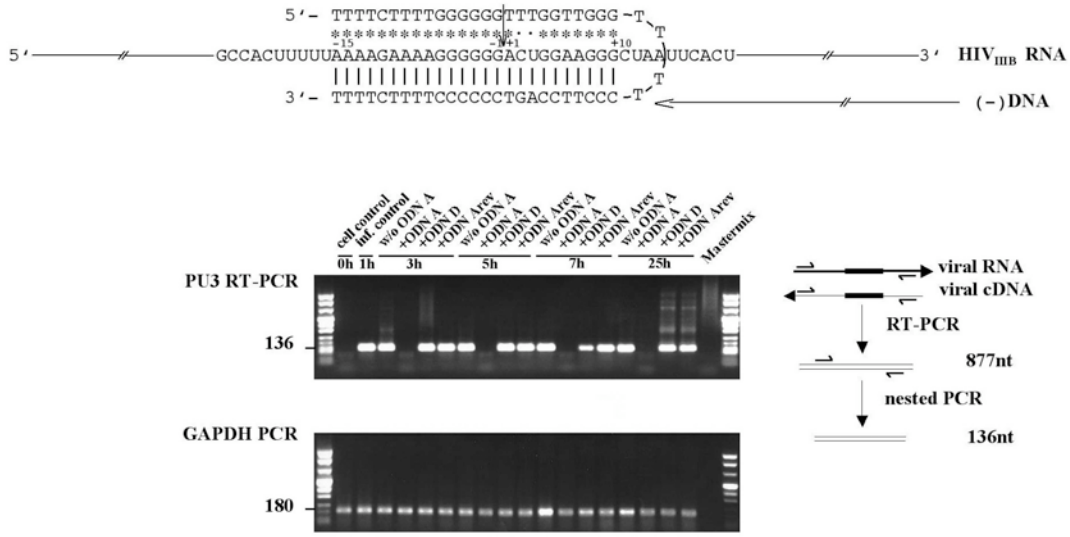


FIG. 5. Predicted secondary structures: (A) TFO A alignment on the target RNA sequence; =, Hoogsteen base pairing; –, Watson–Crick base pairing. Modified nucleotides are marked by asterisks. (B) Folding of TFO A was done by the StemLoop program of the Wisconsin Sequence Analysis Package. (C) Scrambled TFO sequence listed for comparison.

11.5.4 Inhibition of viral replication by oligodeoxynucleotides directed against the polypurine tract of HIV-1

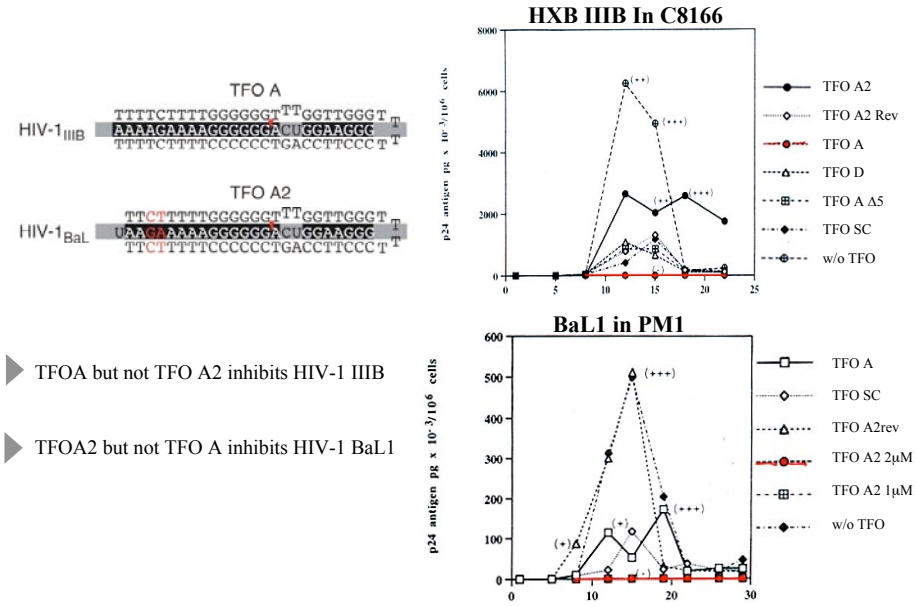
Karin Moelling, Jörg Jendis, Susanne Abels *et al.* (submitted)

ODN against HIV-1_{IIIB}



HIV-1 replication can be inhibited by TFO A.

C8166 cells are infected by HIV-1 for 2h, washed, TFO A is added and the cells are lysed after 3, 5, 7, and 20h as indicated. The nucleic acid is extracted, primers are added and a RT-PCR is performed (see scheme). TFO A inhibits in sequence-specific manner. Single nucleotide changes in the TFO A abrogate the effect. TFO may form intracellular triplexes and prevent HIV-1 replication. TFO A will be tested in SCID-HIV-1 mice through a recent donation.



TFOA but not TFO A2 inhibits HIV-1 IIIB

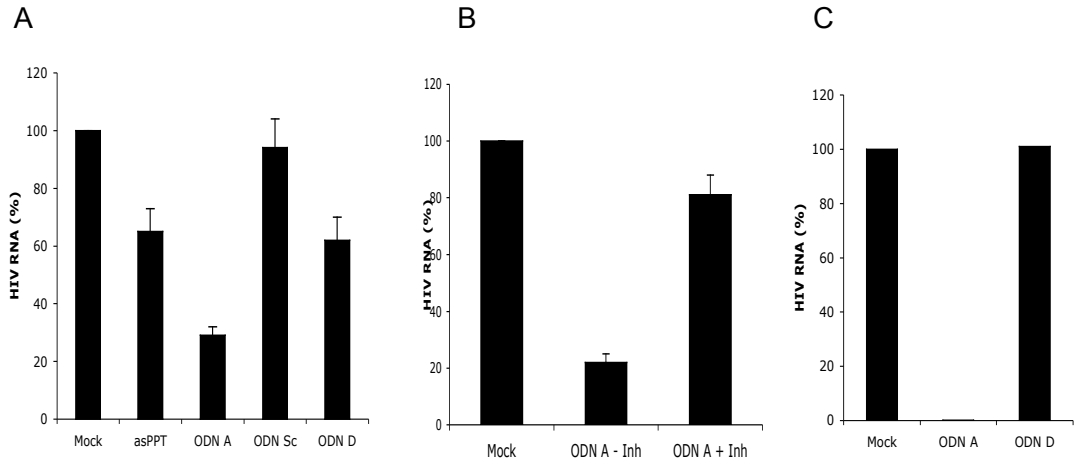
TFOA2 but not TFO A inhibits HIV-1 BaL1

Heterospecificity of TFO A and TFO A2 for HIV-1_{III-B} and HIV-1_{BaLI}

11.5.5 Oligodeoxynucleotide ODN A induces reverse transcriptase-mediated cleavage of HIV RNA and abrogates infectivity of virions

Alexey A. Matskevich, Algirdas Ziogas, Jochen Heinrich, Sandra A. Quast and Karin Moelling (submitted, see cover page)

We describe a novel mechanism of viral RNA silencing by short double-stranded oligodeoxynucleotide A (ODN A, former TFO A) directly in HIV virions. ODN A consists of antisense and passenger strands, and is designed to target polypurine tract of HIV-1 IIIB, the conservative region of viral genome, which is important for virus replication. Activation of HIV reverse transcriptase-RNase H (RT/RNaseH) by ODN A leads to degradation of RNA in viral particles. Illimaquinone, specific inhibitor of RNase H activity of HIV RT/RNaseH abolishes RNA cleavage. The effect of ODN A is sequence-specific and the passenger strand is important, since alteration of the either strands prevents the antiviral activity of ODN. The antiviral effect of ODN A is superior compare to single-stranded antisense oligodeoxynucleotide. ODN A abrogates infectivity of virions preincubated in cell culture medium with ODN A in absence any DNA-carriers or detergents. For elimination of RNA in virions ODN A does not rely on any additional defensive mechanisms, and represents a self-sufficient suicide-inducing system. It attacks the virus directly in cell-free system and thus potentially can serve as alternative drug to reduce virus titer and to prevent infection of newborn cells in the blood of patients infected with HIV.



(**A**) Comparative efficiency of ODNs in virions. ODNs were incubated with intact HIV virions in cell culture medium and analyzed by real-time PCR. (**B**) RNase H inhibitor abolishes ODN A- mediated effects. Permeabilized HIV virions were incubated with RT/RNaseH, ODN A and Illimaquinone, a selective inhibitor of the RNase H activity of HIV RT/RNaseH. Then viral RNA was purified and real-time PCR analysis was performed. (**C**) ODN A abrogates infectivity of HIV virions. HIV virions were incubated with ODNs for 4 hours. C81-66/45 cells were infected with preincubated virions, at 0.1 MOI . RNA was extracted from infected cells and HIV replication was analysed by real-time PCR, 15 days post-infection.

11.5.6 Inhibition of HIV by RNA interference

Martin Schwemmle, Nina Raetzel, Daniel Mayer and Karin Mölling

RNA interference (RNAi) describes the silencing of a target gene by homologous double stranded RNA (dsRNA). The mechanism is phylogenetically highly con-served among species ranging from primitive eukaryotes to mammals. RNAi is a multistep process, in which dsRNA is first cut into 21-23 nt dsRNAs, so called "small interfering RNAs (siRNA)", by DICER. The siRNAs associate with a multicomponent nuclease complex termed RISC ("RNA induced silencing complex") and thus mediate target mRNA destruction.

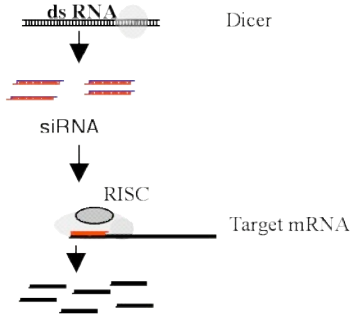


Fig. 1: Processing of siRNAs by DICER and target recognition and degradation by the RISC complex.

To test whether it is possible to prevent HIV replication by RNAi we designed siRNAs directed against the extended polypurine tract region (designated PPT) of HIV1 (Fig. 2A). We could show that such siRNAs are effective in preventing HIV-induced syncytia formation in susceptible GHOST cells (Fig. 2B). Furthermore, we could demonstrate that HIV replication is severely impaired prior to treatment with siRNA directed against PPT (Fig. 2C). In summary, siRNAs directed against the PPT region interfere efficiently with HIV replication. Since the PPT region represents one of the most conserved sequences amongst the majority of HIV1 and SIV strains it might be an attractive candidate for in vivo studies.

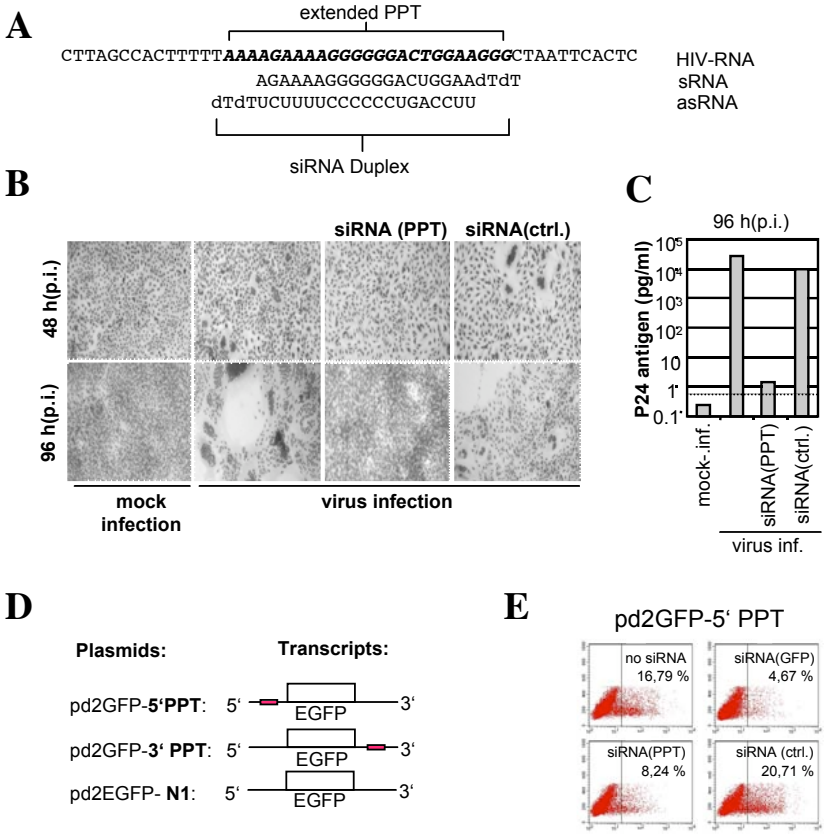


Fig. 2 Inhibition of HIV by RNAi.

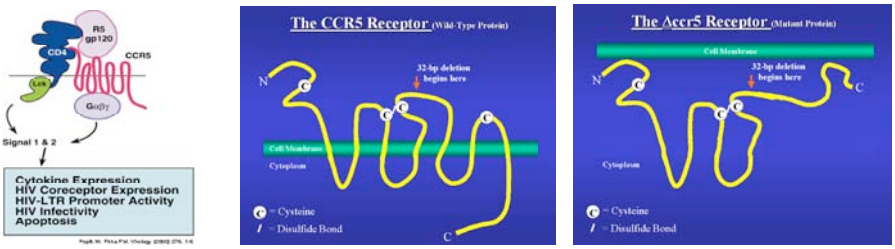
(A) The HIV-1 specific siRNAs used in this study recognize the extended PPT region of the viral HIV-RNA. (B) HIV-induced syncytia formation in GHOST cells after the indicated time and prior to treatment with siRNAs against PPT or control siRNAs. Note that only mock-infected cells or cells treated with siRNA (PPT) show no syncytia formation. (C) P24 antigen levels in the supernatant of HIV1-infected cells. (D) The efficacy of siPPT could also be demonstrated in a transient transfection assay using GFP expression vectors harboring the extended PPT region in either the 5` or 3` untranslated region of the corresponding trans-cripts. (E) Analysis of reporter expression by FACS analysis in the presence or absence of the indicated siRNAs.

11.5.7 The chemokine- and HIV-1 coreceptor CCR5: are interacting proteins regulating receptor function?

Marc Schweneker, André Bachmann, Karin Moelling

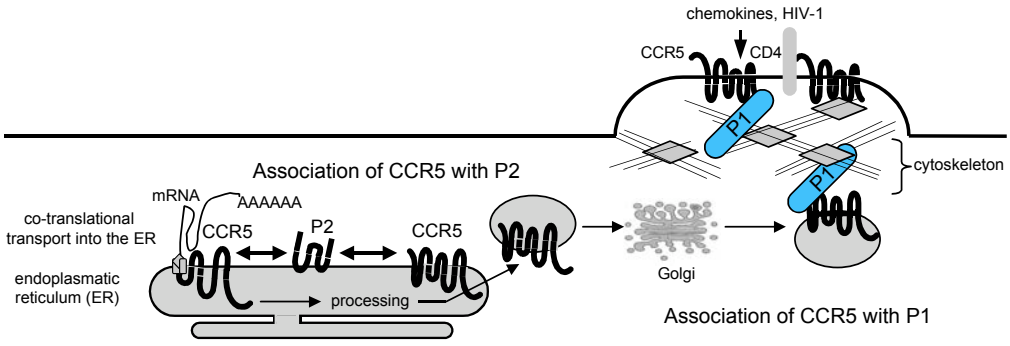
BBRC 325, 751-7 (2004) and FEBS Letters 579, 1751-8 (2005)

In 1984 the CD4 receptor molecule was identified to be essential for HIV-1 infection, but it soon became obvious that further components were required for efficient virus entry. This led to the identification of the two major HIV-1 coreceptors CXCR4 (CXC chemokine receptor 4) and CCR5, both of which belong to the superfamily of seven transmembrane, G-protein coupled chemokine receptors. The key role of CCR5 in HIV-1 transmission is demonstrated by the fact that individuals that express a non-functional mutant of CCR5, termed CCR5Δ32, have been shown to be highly resistant against HIV-1 infection. The severely truncated CCR5Δ32 is not expressed at the cell surface, therby lacking coreceptor function for HIV-1 infection. Furthermore, the C-terminus of CCR5 has been shown to facilitate proper function of the receptor, having significant impact on targeting the receptor to the plasma membrane, on signaling, internalization and intracellular trafficking. Binding of chemokines and also HIV-1 gp120 to CCR5 trigger the activation of a number of different pathways, e.g. the Ras-Raf-MEK-ERK pathway. Other pathways involve focal adhesion kinase or related kinases, Src-related kinases, the PI3Kinase, Rho, PLC, PKA, and PKC. These pathways affect chemotaxis, adhesion, cellular polarization, internalization, recycling or degradation of the receptor. Stimulation of receptor-linked pathways may contribute to dysregulation of cellular functions, and also to the modulation of HIV-1 infection and/or replication or new target cell recruitment.



To further analyze the role of the C-terminus of CCR5 in HIV-1 and chemokine receptor function we used this domain in yeast two-hybrid system to find novel interacting proteins. Two proteins, P1 and P2, were identified. P1 is known to be cytoskeleton-associated, P2 is a novel protein with unknown properties. We analyzed P1, P2 and CCR5 by mutating all three proteins and studied their interaction in yeast and mammalian cells. The characterization of P2 led to the identification of a novel protein family, which members seem to be involved in the regulation of plasma membrane receptor and transporter molecules.

We are further studying these proteins and mutants thereof to elucidate their function, focussing on their biological role in respect to CCR5 as chemokine receptor and in HIV-1 infection.



Model for the interaction of two new proteins, P1 and P2, with CCR5.

Intervirology 2000, **43**

Intervirology
International Journal of Basic and Medical Virology

Viral DNA Vaccines

Guest Editor
Karin Mölling, Zurich

Contents

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Naked DNA for Vaccine or Therapy

Karin Moelling and Jovan Pavlovic

Together with Lina Elzaouk, Nurgül Usluoglu, Jochen Heinrich, Bettina T. Oberle, Jan Schultz, et al.

We have used naked DNA for various approaches. DNA coding for antigens have been used as vaccine against various viral diseases such as HIV, LaCrosse Virus (LACV), Influenza and SARS. The DNA was designed to encode outer surface antigens or internal proteins. In various preclinical animal models we have seen some efficacy however, the amount of protein expressed from DNA is rather low and therefore the vaccination requires additional approaches to improve efficacy. This comprises combinations of DNA with peptides of proteins. Some of these results are summarized below.

Furthermore, we have used DNA encoding with protein Aβ, which is assumed to be the causative agent of alzheimers disease (AD). The DNA vaccination of mice show some reduction of plaque formation and the mice allowed to isolate their spleens for production of monoclone antibodies. These were used for passive immunization where they showed surprising efficacy.

Furthermore, we have used combinations of DNA vaccine with trimeric co-stimuli such as CD40L and observed some entire tumor response.

We also considered alternativ constructs for application of DNA therapy. All these results are summarized in the ffigures below.

We consider DNA not coding for antigen but for a cytokine or chemokine as superior. We have compared about 20 different genes coding for antigens or chemokine for the anti tumor efficacy. Out of these we selected Interleukin-12 (IL-12). This has been used in various preclinical studies and shown efficacy as treatment of tumors by injection into the lesions directly. We were able to demonstrate efficacy of this DNA in therapeutic and prophylactic tumor models as well as therapeutic as a prophylactic against the establishment of metastases. We also analyzed IL-12 DNA in malignant melanoma of grey horses as a second independent preclinical model. On the basis of this, a clinical trial was initiated with 9 malignant melanoma patients (see below). Furthermore, we tried to improve this technology and preclinical models using combinations with IM-10, sFlk-1, gp100 CTL peptide epitopes and more recently with CCL19 and CCL21. Some of these results are summarized in the figures below.

Naked DNA for vaccine and therapy

HIV

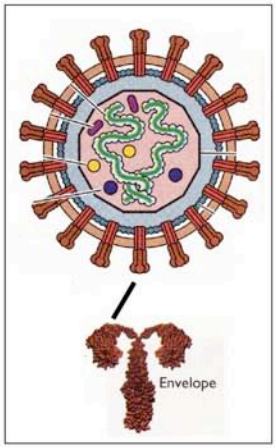
LACV-G1/G2
LACV-N

FLUA-HA
FLUA-NP

$A\beta_{1-42}$

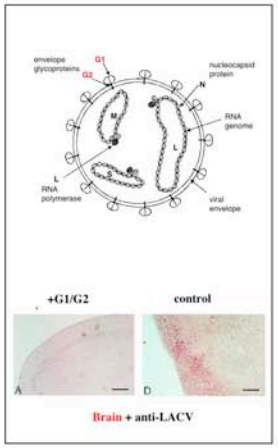
mu-CD40
Hu-gp100

telRL



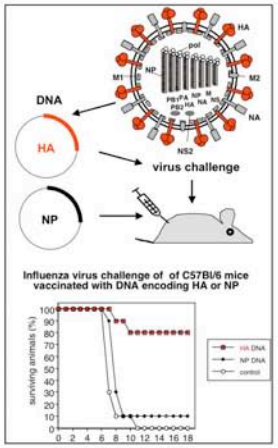
Phase I clinical trial with HIV-1 gp160 plasmid vaccine in HIV-1-infected asymptomatic subjects

Weber et al., Eur J Clin Microbiol Infect Dis 2001, 20(11):800-803



DNA-based vaccine against La Crosse Virus: Protective immune response mediated by neutralizing antibodies and CD4⁺ T cells

Schuh et al., Human Gene Ther 1999, 10:1649-1658

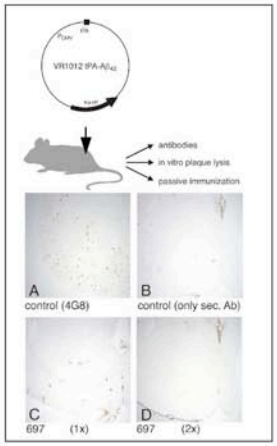


Enhanced protection against viral infection by co-administration of plasmid DNA coding for viral antigen and cytokines in mice

Operschall et al., J Clin Virol 1999, 13:17-27

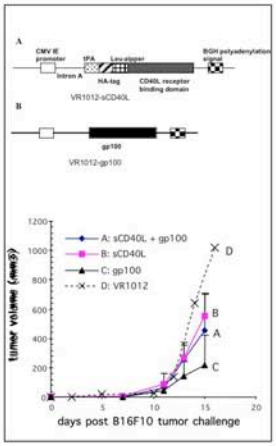
Protection against influenza A virus by DNA vaccination depends on the humoral response and dose of challenge virus

J. Pavlovic et al., Vaccine 2002, 20:1692-1699



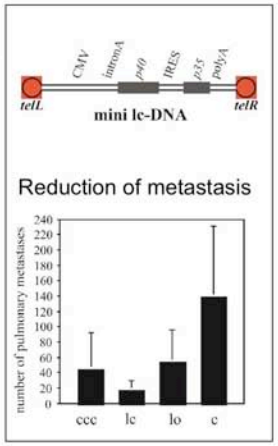
Antibodies from a DNA peptide vaccination decrease the brain amyloid burden in a mouse model of Alzheimer's disease

Schultz et al., J Mol Med 2004, 82(10):706-714



Combination of a DNA vaccine encoding soluble trimeric CD40L with a DNA vaccine for a melanoma specific antigen.

G. Dollenmaier and K. Moelling

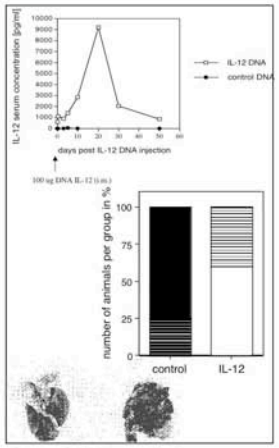


Linear closed mini DNA generated by the procaryotic cleaving joining enzyme TelN is functional in mammalian cells

Heinrich et al., J Mol Med 2002, 80(10):648-654

IL-12 as gene medicine

mu-IL12



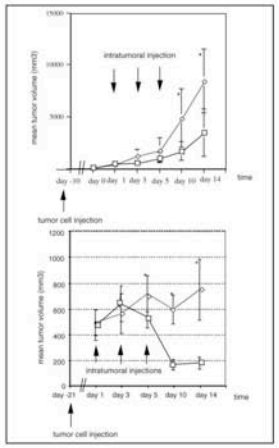
Long-lasting anti-metastatic efficiency of interleukin12 -encoding plasmid DNA

Schultz et al., Human Gene Therapy 1999, 10:407-417

Induction of long-lasting cytokine effect by injection of IL-12 encoding plasmid DNA

Schultz et al., Cancer Gene Therapy 2000, 7(12):1557-1565

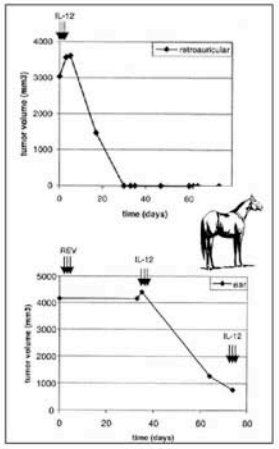
mu-IL12



Tumor regression of human and murine melanoma after intratumoral injection of IL-12 encoding plasmid DNA in mice

Heinzerling et al., Experimental Dermatology 2002, 11:232-240

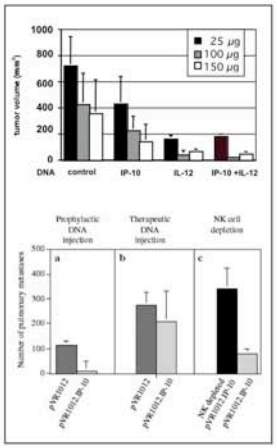
hu-IL-12



Tumor regression induced by intratumoral injection of DNA coding for human interleukin 12 into melanoma metastases in gray horse

Heinzerling et al., J Mol Med 2001, 78:692-702

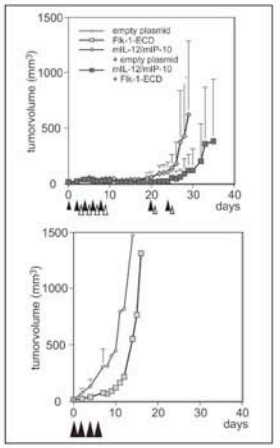
mu-IP-10
mu-IL-12



IP-10-encoding plasmid DNA therapy exhibits anti-tumor and anti-metastatic efficiency

Keyser et al., Experimental Dermatology 2004, 13:380-390

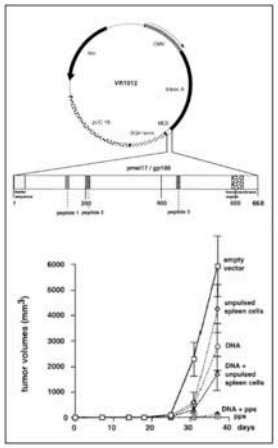
sFlk-1
mu-IL-12
mu-IP10



A combination of plasmid DNAs encoding murine Flk-1 extracellular domain, murine IL-12 and murine IP-10 lead to tumor regression and survival in melanoma-bearing mice

Ladell et al., J Mol Med 2003, 81(4):271-278

hu-gp100



Synergistic effect of a combined DNA and peptidevaccine against gp100 in a malignant melanoma mouse model

Nawrath e t al., J Mol Med 2001, 79:133-142

11.6.1 Anti-tumor activity of mesenchymal stem cells producing murine IL-12 in a mouse melanoma model

Lina Elzaouk, Jovan Pavlovic, Karin Mölling

(submitted)

We report the antitumor effects of intratumoral administration of IL-12-expressing mesenchymal stem cells (MSCs) in comparison to IL-12-encoding DNA. MSCs derived from human adult bone marrow were stably transduced with a retroviral vector coding for the cytokine, IL-12 by expressing the two subunits p40 and p35 from a single gene connected by a flexible linker. These MSC(IL-12) cells have shown a strong effect on retardation of tumor growth in mouse melanoma when applied to established tumors in a therapeutic regimen. This effect was of similar efficiency as that obtained by the naked IL-12 encoding DNA plasmid resulting in long-term survival of the treated mice. The therapeutic effect of the MSC(IL-12) was partly mediated by natural killer (NK) cells, CD4+ and CD8+ T cells as shown by depletion experiments. We demonstrated that these two different strategies can induce a similar therapeutic antitumor efficacy in the murine melanoma tumor model.

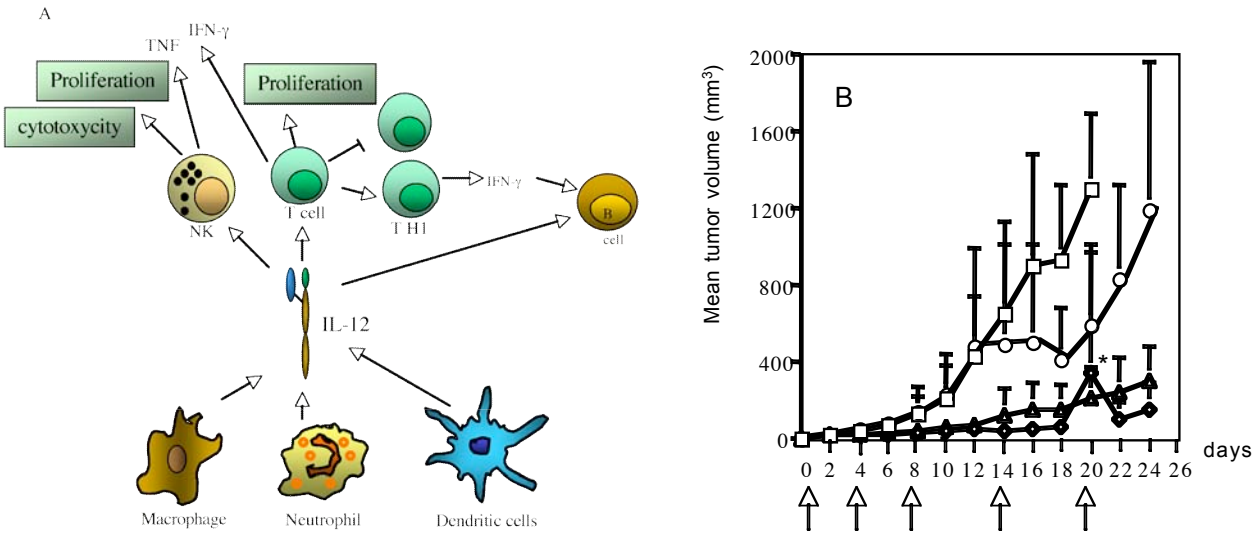


Fig.1 (A) The main physiological producers of IL-12 are macrophages, neutrophils and DC in response to pathogens. The physiologically most important target cells of IL-12 are: NK and T cells for which Il-12 induces proliferation, enhancement of cytotoxicity and the production of cytokines, particularly IFN-γ as well as favouring differentiation to cells that produce type 1 cytokines; and B-cells for which Il-12 directly or through the effects of type-1 cytokines enhances the activation and the production of Th1 associated classes of Ig.

(B) Effect of MSC(IL-12) on tumor growth. Groups of 8 mice were intratumoraly injected with 100 ug IL-12 DNA (Δ), or $7.5x10^5$ MSC(IL-12) (÷) or as controls $7.5x10^5$ MSCs (O) or 100 ul PBS (□) on days 0, 4, 8, 14, and 20.

11.6.2 Synergistic anti-tumor activity elicited by vaccination with IL-12-encoding plasmid DNA in combination with the chemokine CCL21 in vivo

Lina Elzaouk, Jovan Pavlovic, Karin Mölling

(submitted)

The chemokine CCL21 plays an important role in attracting naive T cells and immature dendritic cells (DCs) from the periphery to secondary lymph organs associated with sites of inflammation or tumors. This feature, recruiting professional antigen-presenting DCs and T lymphocytes makes CCL21 a pime candidate for tumor therapy. In the present study we demonstrate that intratumoral injection of recCCL21 protein or DNA encoding CCL21 exerted an antitumor effect against malignant melanoma and renal cell carcinoma in syngeneic mouse models. Furthermore, we observed a pronounced synergistic antitumor activity in the mouse melanoma model when CCL21 was applied in combination with DNA encoding IL-12. Surprisingly, treatment with a combination of CCL21 with DNA encoding the tumor-associated antigen gp100 was not effective, while co-application of DNA encoding IL-12 with DNA encoding hgp100 showed a very strong antitumor effect.

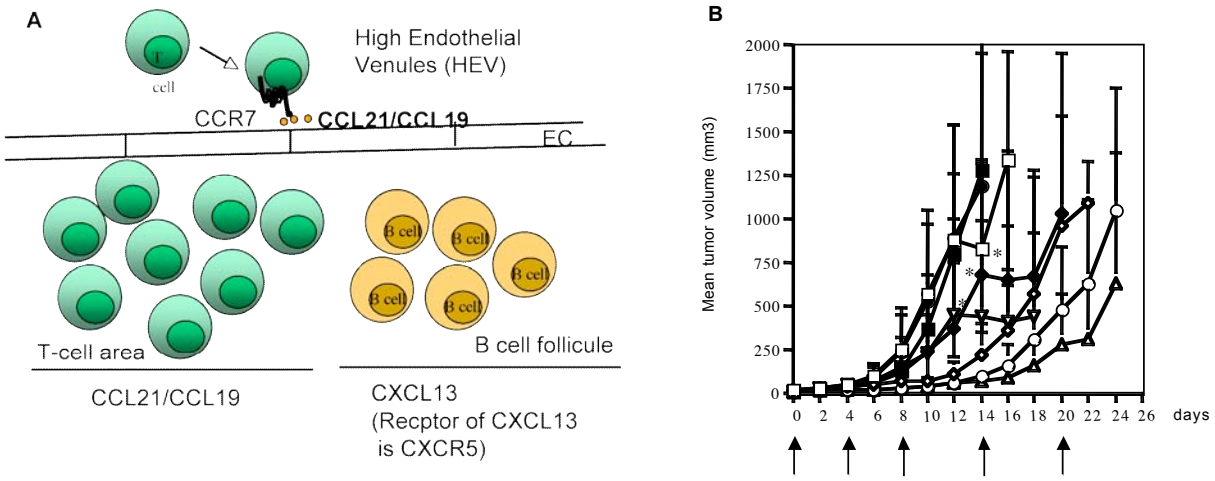


Fig.1 (A) Lymphocytes entering the so called high endothelial venules, tether and roll along the endothelium in direction of the blood flow. During this rolling, chemokines presented on the endothelial surface bind to lymphocyte-expressed chemokine receptor, such CCR7 and CXCR4 and CXCR5. **(B)** Time course of B16F10 melanoma tumor growth in C57BL/6 mice treated by administration of muCCL21 DNA and hgp100 DNA. Tumors were established by s.c. inoculation of $1x10^5$ B16F10 cells into the right hind flank. When a mean tumor volume of 15-20 mm^3 was reached (day 0), treatment was started. Groups of 7-9 mice were i.t. injected with 100ug control DNA (□), 100 ug muIL-12 DNA (÷), 0.4 ug recCCL21 protein (●), 100 ug hgp100 DNA (∇), combination of 100 ug muIL-12 DNA with either 0.4 ug recCCL21 protein (O) or 100 ug muCCL21 DNA (Δ), combination of 0.4 ug recCCL21 protein with 100 ug hgp100 DNA (■), and combination of 0.4 ug recCCL21 protein with 100 ug muIL-12 DNA and 100 ug hgp100 DNA (♦) on days 0, 4, 8, 14, and 20

11.6.3 Therapy of Tuberculosis in Mice by DNA Vaccination

Nature, 400, 269-271 (1999)

29. Coward, P. *et al.* Controlling signaling with a specifically designed Gi-coupled receptor. *Proc. Natl Acad. Sci. USA* **95**, 352–357 (1998).
30. Winzer-Serhan, U. H., Broide, R. S., Chen, Y. & Leslie, F. M. Highly-sensitive radioactive in situ hybridization using full-length hydrolyzed riboprobes to detect alpha 2 adrenoreceptor subtype mRNAs in adult and developing brain. *Brain Res. Brain Res. Protoc.* **3**, 229–241 (1999).

Acknowledgements. We thank S. Rattan and S. Merten for technical assistance; C. Li for making stable cell lines at the initial stage; Y. Chen for *in situ* hybridization; V. H. Cao and A. Henschen–Edman for mass spectrometry and sequence analysis; F. Monsma and R. Henningsen for chimaeric G-protein constructs; D. Piomelli and Q.-Y. Zhou for critical reading of the manuscript; and B. O'Dowd for discussions on orphan receptors. This work was supported by a grant from Hoffman-La Roche and by the Eric L. and Lila D. Nelson Chair in Neuropharmacology.

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Therapy of tuberculosis in mice by DNA vaccination

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Mycobacterium tuberculosis **continues to kill about 3 million people every year[1], more than any other single infectious agent. This is attributed primarily to an inadequate immune response towards infecting bacteria, which suffer growth inhibition rather than death and subsequently multiply catastrophically. Although the bacillus Calmette–Guérin (BCG) vaccine is widely used, it has major limitations as a preventative measure[2]. In addition, effective treatment requires that patients take large doses of antibacterial drug combinations for at least 6 months after diagnosis[3], which is difficult to achieve in many parts of the world and is further restricted by the emergence of multidrug-resistant strains of *M. tuberculosis*. In these circumstances, immunotherapy to boost the efficiency of the immune system in infected patients could be a valuable adjunct to antibacterial chemotherapy[4]. Here we show in mice that DNA vaccines, initially designed to prevent infection, can also have a pronounced therapeutic action. In heavily infected mice, DNA vaccinations can switch the immune response from one that is relatively inefficient and gives bacterial stasis to one that kills bacteria. Application of such immunotherapy in conjunction with conventional chemotherapeutic antibacterial drugs might result in faster or more certain cure of the disease in humans.**

DNA vaccination protects mice against subsequent challenge with tuberculosis[5–7] by establishing a cellular immune response that is dominated by antigen-specific T lymphocytes that both produce interferon-γ (IFN-γ) and are cytotoxic towards infected cells (a type-1 cellular immune response). Both of these functions are probably required for maximally effective antimycobacterial immunity in mice[8] and in humans[9,10]. These responses are particularly favoured by DNA vaccines[11]. In contrast, antigen-specific T cells that produce interleukin-4 (IL-4) and are not cytotoxic (a type-2 cellular immune response) are abundant during infection with *M. tuberculosis*[12,13]. Such type-2 responses do not contribute to protection[8] and so a shift in the balance towards type-1 responses might be beneficial. Furthermore, because effective immunity to tuberculosis probably depends on possessing appropriate responses to multiple antigens, a broad shift in the phenotype of T cells

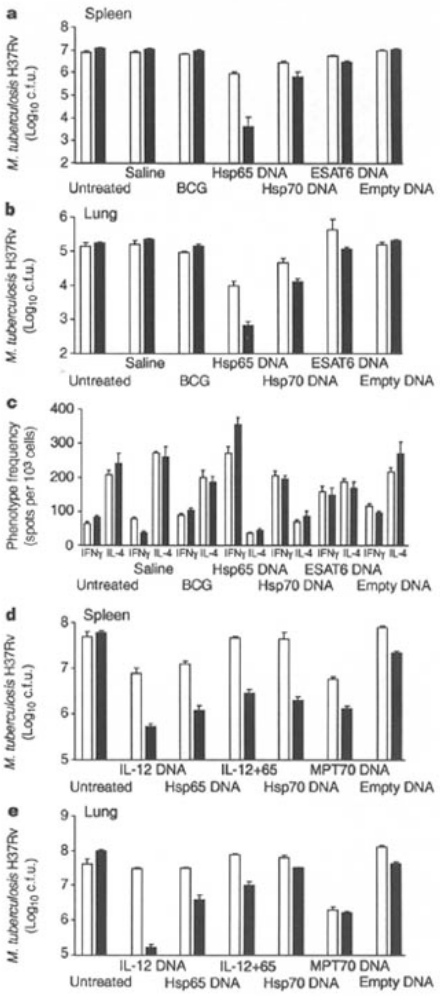


Figure 1 Therapeutic effects of DNA vaccination against an established *M. tuberculosis* H37Rv infection. **a–c**, Declining bacterial numbers and altered T-cell profiles after treatment with DNA encoding antigen Hsp65. Treatment commenced 8 weeks after intravenous injection of 5×10^5 live bacteria, when there were about 2×10^6 and 8×10^4 bacteria in spleens and lungs, respectively. Four intramuscular injections of plasmid DNA were given at 2-week intervals. The numbers of live bacteria in spleen (**a**) and in lung (**b**) 2 months (open bars) and 5 months (filled bars) after treatment commenced are shown as mean ± standard deviation (s.d.) ($n = 5$). Frequencies of lymph node cells (**c**) producing IFN-γ or IL-4 at 2 months (open bars) or at 5 months (solid bars) after treatment commenced were assayed by ELISPOT. Results are mean ± s.d. from triplicate determinations on cells pooled from groups of five mice. The effects of Hsp65 and Hsp70 DNAs were highly significant in both organs compared with empty plasmid or untreated controls at both time points (Student's t-tests, $P < 0.001$). **d**, **e**, Treatment with DNA expressing either antigen MPT70 or IL-12 also caused bacterial decline in spleen (**d**) and lung (**e**). Treatment commenced 8 weeks after intraperitoneal injection of 10^6 live bacteria when there were about 3×10^7 and 4×10^7 bacteria in spleen and lung, respectively. Counts of bacteria at 8 weeks (open bars) and 11 weeks (solid bars) after commencement of treatment are shown as mean ± s.d. ($n = 5$). The effects of IL-12, MPT70 and Hsp65 DNAs were highly significant in both organs at 11 weeks compared with either empty DNA or untreated controls (Student's *t*-tests, $P < 0.001$).

11.6.4 Development of an immunotherapy for breast cancer based on dendritic cells by developing and comparing different types of tumor specific immunogens

Jovan Pavlovic, Karin Moelling, Nurgül Usluoglu (IMV, Zurich) together with Joyce Taylor-Papadimitrou (ICRF, London, United Kingdom), Thomas Noll (Forschungszentrum Juelich, Juelich, Germany), Jaques Bartholeyns (IDM, Paris, France) Yvette van Kooyk (University of Amsterdam, Amsterdam, The Netherlands), Gerard Bos (University of Maastricht, Maastricht, The Netherlands), Henrik Clausen (University of Copenhagen, Copenhagen, Denmark), Gunnar Hannson (University of Goteborg, Goteborg, Sweden)

Dendritic cells (DC) are the most potent antigen-presenting cells for the initiation of antigen-specific immune responses. In addition to their ability to efficiently aquire and process antigens, DC express high levels of MHC class I and class II molecules as well as costimulatory molecules essential in antigen presentation (Fig.2). Therefore dendritic cells are capable of recruiting the multiple components of the immune system.

This project aims for the development of an effective immunotherapy for breast cancer, based on dendritic cell vaccines.The primary task of this project was (i) to evaluate several delivery methods for the tumor-associated antigen mucin1 (MUC1) to human monocyte-derived DCs. And (ii) to assess the immune therapeutic potential of these MUC1 loaded DCs in vitro. MUC1 is a cell surface protein that is overexpressed and abberantly glycoylated in a high proportion of mamma carcinoma. We have evaluated several viral and non-viral delivery systems and observed that transduction of human DCs with a lentiviral vector encoding MUC1 was most efficient Lentiviral transduction lead to efficient ectopic expression of MUC1 on the cell surface of DCs (Fig 2). We currently evaluate the extent of MUC1 antigen presentation of transduced DCs, and evaluate the immune response using patients T-cells *in vitro.*

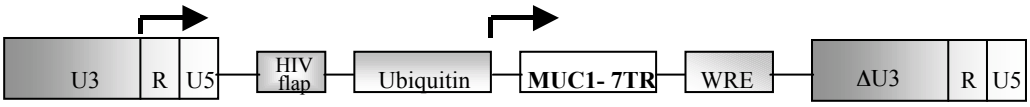


Fig.1 HIV -1 based lentiviral vector expressing the cancer antigen MUC1

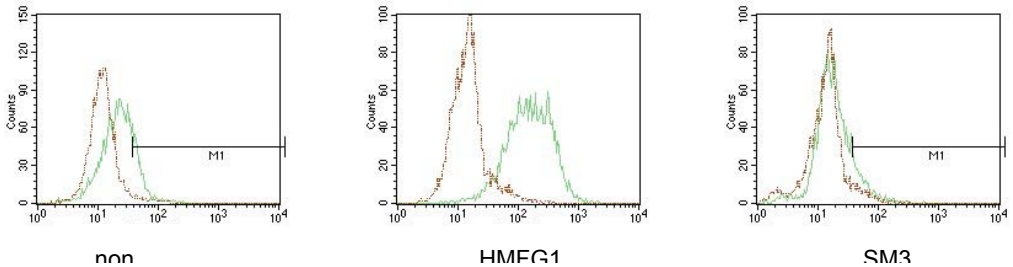


Fig.2 FACS analysis of DC transduced with the lentiviral vector expressing MUCI antigen using the HMFG1 and SM3 antibodies

11.6.5 A prime boost strategy for immunotherapy of breast and ovarian cancer

Jovan Pavlovic, Karin Moelling, Nurgül Usluoglu (IMV, Zurich) together with Joyce Taylor-Papadimitriou (Cancer Research UK, London, United Kingdom), Thomas Noll (Forschungszentrum Juelich, Germany), Gunnar Hannson, Thommy Nilsson (University of Goteborg, Sweden), Jan Kihlberg (Umea University, Sweden), Henrik Clausen (University of Copenhagen, Denmark), Marianna Nuti (University of Rome, Italy), and David Snary (ICRT, London, United Kingdom)

The goal of this study is evaluate and develop an immune therapy protocol against mamma carcinoma that is directed against the tumor-associated antigen mucin1 (MUC1). For this purpose the know-how of clinical groups, reseach groups in the field of cancer immunology and groups which are experts on chemical synthesis of glycopeptides and large scale cell culture expression of glycoproteins were combined. The MUC1is a transmembrane protein expressed on epithelial cells and has a great potential as a cancer vaccine because it is overexpressed and aberrantly glycosylated in more than 90 % of breast and ovarian cancers as well as in a proportion of other carcinomas. The extracellular region of MUC1 consists mainly of a variable number of tandem repeats made up of 20 amino acids that show a complex pattern of glycosylation. Since both humoral and cellular responses to MUC1 have been found in cancer patients, an appropriately scheduled active immunization with MUC1-based immunogens should improve this immune response to make it effective in tumor rejection.

The vaccination strategy employed in ths project is based on a prime-boost regimen that includes priming of an humoral and cellular immune response by vaccination with plasmid DNA encoding MUC1 followed by a booster immunization with MUC1-derived glycopeptides or glycoproteins. Our contribution to this vaccine project is to optimize the priming of the immune response against MUC1 by evaluating several plasmid DNA-based expression systems including a semliki forest virus (SFV)-derived replicon in preclinical mouse tumor models.

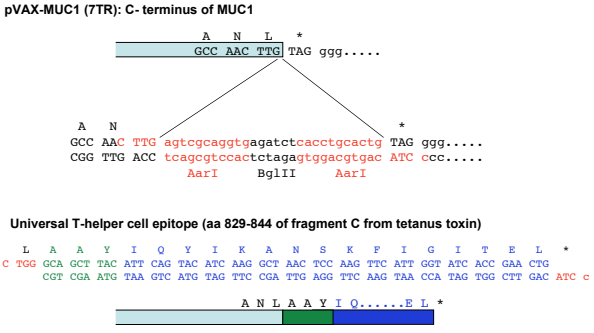


Fig. 1. Construction of a plasmid DNA encoding MUC1 fused to a universal T-helper epitope derived from the C fragment of tetanus toxin (ttc)

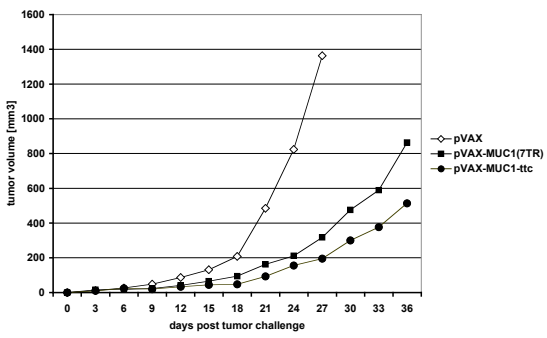


Fig. 2. Inhibition of B16F10 tumor growth by vaccination of syngeneic C57BL/6 mice with plasmid DNA encoding the MUC1-ttc fusion protein.

11.6.6 Development of a DNA vaccine against SARS-CoV

Bettina T. Oberle, Karin Mölling, Jovan Pavlovic (Thesis ongoing)

Severe acute respiratory syndrome (SARS) has a high potential of pandemic spread and it is of worldwide interest to control the new aetiologic agent SARS-CoV with a successful vaccination. In order to develop a potent vaccine against SARS-CoV, we examine a naked DNA immunization, which offers many advantages compared to traditional vaccines. To strengthen DNA immunization by improving viral spike (S) protein presentation we tested several constructs based on the viral S protein, with substitutions of the signal sequence and the transmembrane and cytoplasmic tail, either alone or in combination. To improve extracellular presentation of the viral S protein, we replaced its TM and CP domains with a GPI anchor sequence (sSG). GPI-anchor proteins have been described to optimise the presentation of proteins to immune responsive cells through accumulation in lipid rafts. Consistently, we show that the sSG protein, but not S protein without GPI anchor, is targeted to lipid rafts. In an alternative approach, we substituted the wild type signal sequence of the S protein with the haemagglutinin leader (HA) of influenza A/PR/8/34. We show that the S protein with the two modifications was transported more efficiently to the cell surface. To validate improved presentation of S protein and immune response in vivo, we injected the constructs into C57Bl/6 mice. All constructs produced immune response but with variations in their specificity as evidenced by ELISA. Expression of S protein fused with either HA-leader or GPI anchor improved immune response in a SARS-IIFT test, compared to S protein. Moreover we demonstrate appearance of neutralizing antibodies in immunized mice.

In summary, fusion of a GPI-anchor to the viral S protein leads to enhanced presentation, resulting in stronger antigen stimulation. We propose that forced targeting of antigens to membrane rafts may improve DNA vaccination strategies in general.

Modifications of the Spike for enhanced immune response: Introduction of the signal sequence of influenza hemagglutinin (HA) and a GPI-anchor in place of the transmembrane region

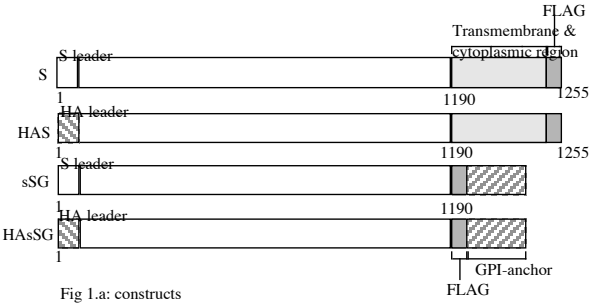
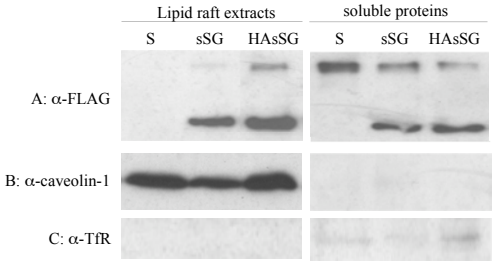
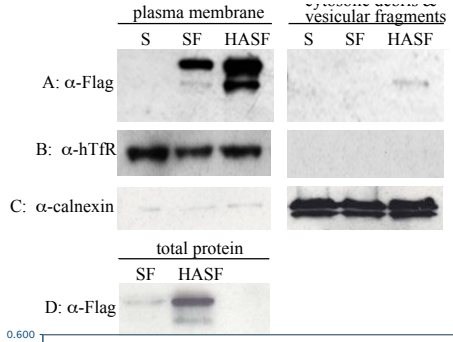


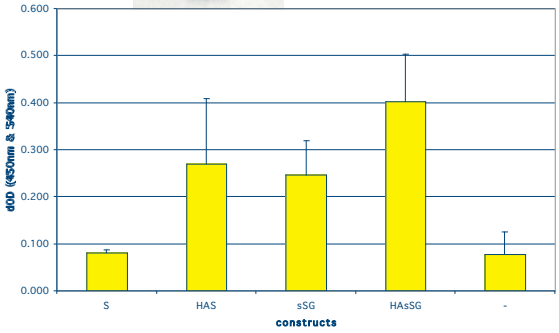
Fig 1.a: constructs



Western blot analysis to identify the localization of the S-GPI fusion proteins: sSG and HasSG but not S localize in Lipid rafts. Caveolin 1 is protein localizing in lipid rafts (positive control), whereas the transferrin receptor is a plasma membrane marker.

Western blot to show expression and localization of the S protein and the HAS chimera at the plasma membrane.





Production of spike specific antibodies assessed by ELISA after 4th immunization. Soluble SF served as coating material (1:100). The dOD values were substracted from the values of the non-immunized

11.7 Clinical Trial: Development of a DNA Vaccine against HIV-1

1990-1993	**Initiation and research on DNA vaccine against HIV-1** Joint-appointment of: Karin Moelling, Head of Research Group at MPI for Molecular Genetics, Berlin, D & Director of Molecular and Cellular Biology, Apollon Inc, USA Responsibility: Development of a clinical approval plasmid DNA construct in collaboration with the FDA, Bethesda, MD, USA

December 1993	**Meeting at the Institute of Medical Virology** Presentation of the project by the CEO of Apollon Inc., Dr. V. Zurawski First evaluation for the possibility of a Swiss trial Invited guests: Prof. Dr. C. Weissmann, Prof. Dr. H. Diggelmann, Head of the SKBS, Prof. Dr. R. Lüthy, USZ

May 1994	**Scientific presentation on DNA vaccine against HIV-1** by Dr. D. Weiner, University of Pennsylvania, Philadelphia, USA

May 1995	**Approval of a phase I clinical trial by the FDA, Bethesda, USA**

June 1995	**First volunteer immunized in Philadelphia, USA**

September 1995	**Approval of the Swiss trial by the SKBS** Scientific coordinator: Prof. Dr. K. Moelling Clinical investigator: Prof. Dr. R. Lüthy

March 1996	**Four volunteers immunized at the University Hospital in Zurich**

March 1997	**Termination of Phase I/II trial in Zürich and Philadelphia**

1998/2002	**Follow-up of patients in Zurich for safety parameters**

Publication: Weber R, Bossart W, Cone R, Luethy R, and Moelling K. Phase I clinical trial with HIV-1 gp160 plasmid vaccine in HIV-1-infected asymptomatic subjects (2001). *Eur. J. Clin. Microbiol. Infect. Dis. 20, 800-803*

11.8 Investigator-driven Phase I Clinical Trial "Immunotherapy in Patients with Metastatic Malignant Melanoma by Intratumoral Injection of Naked Plasmid DNA Encoding Human Interleukin 12"

K. Moelling (Scientific coordinator and Sponsor)

1994 - 1999 Preclinical studies with then different cytokine-encoding or tumor-associated antigen-coding DNAs in retroviral vectors or as naked DNA. Various animal models.

- B16-F10 mouse melanoma model and mouse melanoma metastasis in the lung
- Colon cancer, pancreatic cancer, Lewis lung lymphoma, etc.
- Grey horses with naturally occurring melanomas

Publications on preclinical results

Schultz, J., Pavlovic, J., Strack, B., Nawrath, M. and Moelling, K.: Long-lasting anti-metastatic efficiency of IL-12-encoding plasmid DNA. Human Gene Therapy 10, 407-417 (1999).

Schultz, J.,Heinzerling, L. Pavlovic, J. and Moelling, K.: Induction of long-lasting cytokine cascade by injection of IL-12 encoding plasmid DNA. Cancer Gene Therapy, 7, 1557-1565 (2000).

Heinzerling, L., Feige, K., Rieder, S., Akens, M., Dummer, R., Stranzinger, G., Moelling, K.,: Tumor regression induced by intratumoral injection of DNA coding for human interleukin 12 into melanoma metastases in gray horses. Journal of Molecular Medicine, 78, 692-702 (2001).

Publications on clinical results

Heinzerling, L., Burg, G., Dummer, R., Maier, T., Oberholzer, P.A., Schultz, J., Elzaouk, L., Pavlovic, J. and Moelling K.: Intratumoral injection of DNA encoding human interleukin 12 into patients with metastatic melanoma: Clinical efficacy. Human Gene Therapy 16, 35-48 (2005).

	Design of Clinical Protocol, Investigator's Brochure, Patient's consent
	1st SKBS meeting: Presentation of preclinical results in mice. Request by SKBS for more results.
18.6.1999	Written approval for human use of IL-12 DNA by Hoffmann-La Roche (after 2 years of negotiation)
24.11.1999	"Pre-IND" meeting organized by K. Moelling with BERNA and BAG, Bern (Glueck, Zurbriggen, Paroz, Struck, Lambert, Pavlovic, Moelling), no criticism on protocol, no decision on production.
Dec. 1999	1st DNA production ("in the spirit of GMP", US and UK standard) supported by KTI project.
3.3.2000	Meeting with Prof. G. Burg, USZ, Dept. of Dermatology, agreement as clinical investigator.
14.3.2000	2nd presentation to SKBS, Bern. Reject of 1st GMP production and request for destruction.
29.3.2000	Approval of clinical protocol Ref. No. GT-2000011.
26.5.2000	Application to Kantonale Ethics Committee (KEK), No. 383
13.7.2000	Approval by Ethics Committee
11.7.2000	Clinical study notification, Bern, and approval
Aug. 2000	2nd GMP-DNA Production by BERNA Co., Bern
Oct. 2000	Approval by BAG for GMP-DNA production 100 mg and tox. study
16.10.2000	Financing of GMP-DNA production by SNF, NFP37
Dec. 2000	Begin of clinical study
Sept.. 2002	Treatment last (9th) patient
Febr. 2002	Compassionate trial Charité, UKRV, Berlin
Nov. 2003	Final report to Swissmedic on the trial with positive evaluation
Jan. 2005	Publication of clinical results in Human Gene Therapy (see above)
	Compassionate trial at USZ, Zurich
2005/2006	Continuation of clinical trial

HUMAN GENE THERAPY 16:35–48 (January 2005)

11.8.1

Intratumoral Injection of DNA Encoding Human Interleukin 12 into Patients with Metastatic Melanoma: Clinical Efficacy

LUCIE HEINZERLING,[1–3] GÜNTER BURG,[1] REINHARD DUMMER,[1] TANJA MAIER,[1,4] PATRICK A. OBERHOLZER,[1] JAN SCHULTZ,[2] LINA ELZAOUK,[2] JOVAN PAVLOVIC,[2] and KARIN MOELLING[2]

ABSTRACT

Plasmid DNA encoding human interleukin 12 (IL-12) was produced under GMP conditions and injected into lesions of nine patients with malignant melanoma (stage IV) previously treated with both standard and nonstandard therapies. The treatment was based on efficacy in preclinical studies with melanoma in mice and gray horses. The DNA was applied in cycles, three injections per cycle, for up to seven cycles. Three therapy arms comprised low (2 mg), medium (4 mg), and high (10 to 20 mg) amounts of total DNA. The therapy was well tolerated. Three of nine patients experienced a clinical response: two stable disease and one complete remission. One patient receiving a low dose of DNA experienced a long-lasting stabilization of the disease for more than 3 years, whereas the other two responders received high doses of DNA. All patients but one (patient 9) experienced a transient response at the intratumoral injection site. Immunohistochemical staining of responder sections showed local reduction of angiogenesis and lymphocyte infiltrations. All patients, in particular the clinical and local responders (patients 3, 7, and 8), exhibited an antigen-specific immune response against MAGE-1 and MART-1, which in some cases preexisted. Biopsies of responders showed some increase in IL-12, IP-10, and IFN-γ. Serum levels revealed fluctuations. The results show that intratumoral injection of DNA produced some beneficial clinical effect. DNA encoding a cytokine may be useful as a therapeutic or adjuvant against various human cancers.

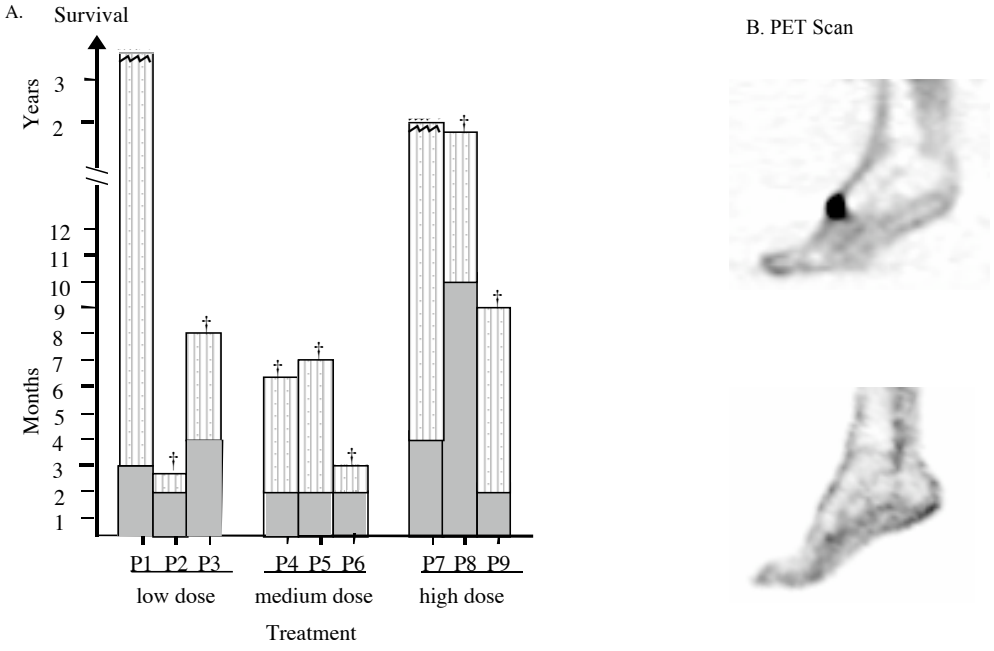


Fig.**1** Survival time of the individual patients after beginning of therapy. The grey bars indicate the duration of treatment, the striped bars survival after end of therapy (A). Effect of IL-12 DNA treatment on the development of melanoma metastases PET scan of a histologically verified melanoma metastasis of the foot before IL-12 DNA treatment of P7 (B).

11.9 Research Cooperation Agreements, Partnerships

11.9.1 Overview of the Research Cooperations

- Collaborations of the unit with other research institutions in Switzerland and abroad

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Yale University School of Medicine
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Campus address: 300 Cedar Street, TAC-S141B
New Haven, CT 06520
Tel: 001 203 785 6319, jonathan.bogan@yale.edu

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E-mail: http://mayoresearch.mayo.edu/mayo/research/staff/cattaneo_r.cfm

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Thielallee 63, D-14195 Berlin
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Dr. Ronald Frank **Peptide**
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Mascheroder Weg 1, D - 38124 Braunschweig
Tel.: 0049 531 618 17 20, rfr@gbf.de

Prof. Dr. J.M. Lehn **Protein recognition, drug-design**
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Tel.: 0033 1400 319 32, lehn@isis.u-strasbg.fr, lehn@idf.inserm.fr

Prof. Dr. C. Nicolau, **Antikrebstherapie**
Visiting Professor of Medicine at the Tufts University at Boston
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Prof. M. Paul **Akt-Raf crosstalk**
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Garystrasse 5, D-13195 Berlin

Cheryl A. Stoddart Ph.D. **SCID Mäuse für HIV Inhibitor**
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Prof. Dr. med. Brigitte Stöver **Clinical Trial**
Abteilung Pädiatrische Radiologie, Klinik für Strahlenheilkunde
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Joyce Taylor-Papadimitriou **Entwicklung einer Prime Boost Strategie für Immunotherapie gegen Brustkrebs**
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Kyonggeun.Yoon@jefferson.edu

11.9.2 Expenditure of Third Party Funds ("Total Costs" in SAP)

- Die Finanzzahlen werden von der Finanzabteilung der Universität bereitgestellt. Der Bezug der Angaben von der Finanzabteilung wird von der Evaluationsstelle organisiert. Aufwendungen pro Jahr („Total Kosten" gemäss SAP). Im Falle von mehreren Gesuchstellenden nur den eigenen Anteil aufführen. Bitte keine Doppelzählungen.

Tabelle 8 : Expenditure of Third Party Funds for Teaching and Research

Source of Funds	in 1000 Fr.* 2000	2001	2002	2003	2004	in % 2004
SNF Projects						
1. SNF Project 246235 Moelling Karin	55.9	-	-	-	-	
2. SNF Project 246280 Moelling Karin	53.8	-	-	-	-	
3. SNF Project 246317 Moelling Karin	68.2	-	-	-	-	
4. SNF Project 246889 Moelling Karin	-	10.4	59.2	184.1	65.1	
5. SNF Project 246926 Moelling Karin	12.6	15.8	138.6	165.6	-	
6. SNF Project 246945 Moelling Karin	-	120.0	-	-	-	
7. SNF Project 44162101 Radziwill Gerald	-	-	-	-	35.3	
Subtotal I	**190.5**	**146.2**	**197.8**	**349.7**	**100.4**	**12.2**
Other competitive programs**						
1. Project 230194 Bossart Walter	3.7	15.3	14.0	3.2	10.7	
2. Project 230827 Moelling Karin	0.3	55.6	9.8	-	-	
3. Project 232001 Moelling Karin	-	-	7.5	20.7	-	
4. Project 232009 Moelling Karin	25.1	-	-	-	-	
5. Project 232091 Moelling Karin	70.6	78.1	-	-	-	
6. Project 232137 Moelling Karin	45.1	60.6	26.2	112.4	88.8	
7. Project 232265 Moelling Karin	69.6	-	-	-	-	
8. Project 238078 Moelling Karin	12.8	31.7	13.9	36.8	54.3	
9. Project 238109 Moelling Karin	3.1	12.7	52.1	77.8	-	
10. Project 238178 Moelling Karin	2.0	145.7	197.7	94.9	-	
11. Project	-	-	-	14.2	20.1	

43162109 Moelling Karin						
12. Project 34162108 Moelling Karin	-	-	-	38.4	204.3	
13. Project 34162109 Moelling Karin	-	-	-	10.3	152.6	
14. Project 34162110 Moelling Karin	-	-	-	-	32.9	
Subtotal II	**232.3**	**399.7**	**321.2**	**408.9**	**563.8**	**68.3**
Non-competitive programs ***						
1. Project 230139 Moelling Karin	71.8	62.4	139.7	156.5	161.3	
Subtotal III	**71.8**	**62.4**	**139.7**	**156.5**	**161.3**	**19.5**
Total Third Party Funds	**494.6**	**608.3**	**658.7**	**915.1**	**825.5**	**100%**
BMBF, "Leitprojekt Molekulare Medizin" (D)				1999 – 2003: 1.4 Mio DM		

* Expenditures per year. For projects with multiple research grant applicants, list only the funds granted to your part of the project. Please do not enter funds twice.

** KTI; COST; EU Research Program; University of Zurich Research Credit; other.

*** gifts, bequests, donations from foundations, contract research, other.

11.9.3 Publications, 2000 - 2004

➢ Vollständige Publikationslisten z.B. in Anhang. Publikationsliste des/der Lehrstuhlinhabers/in und der Mitarbeitenden getrennt nach Erscheinungsjahr und folgenden Publikationsarten:

Articles in Academic/Scientific Journals

➢ (Bitte Publikationsliste nach folgenden Arten gruppieren – allenfalls den Usanzen des Fachs anzupassen.)

- *Originalarbeiten*
  - *In peer reviewed journals / in referierten Zeitschriften*

2000

Heinicke, T., Radziwill, G., Nawrath, M., Rommel, C., Pavlovic, J. and **Moelling, K.:** Retroviral gene transfer of dominant negative Raf-1 mutants suppresses Ha-ras-induced transformation and delays tumor formation. *Cancer Gene Therapy* **7**, 697-706 (2000).

Heinrich, J., Bosse, M., Eickhoff, H., Nietfeld, W., Reinhardt, R., Lehrach, H. and **Moelling, K.**: Induction of putative tumor-suppressing genes in Rat-1 fibroblasts by oncogenic Raf-1 as evidenced by robot-assisted subtractive hybridization. *Journal of Molecular Medicine* **78**, 380-388 (2000).

Nawrath, M., Pavlovic, J. and **Moelling, K.:** Inhibition of human tumor formation by targeting a repressor Myb-KRAB to DNA. *Cancer Gene Therapy* **7**, 963-972 (2000).

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Manuscripts not yet published

Moelling, K., Abels, S., Jendis, J, Ziogas, A., Matskevich, A, Heinrich, J. Silencing of HIV RNA by a hairpin-loop DNA (submitted).
Fritzius, Th., Burkard, G., Haas, E., Heinrich, J., Schweneker, M., Bosse, M., Zimmermann, S., Frey, A.D., **Moelling, K.** WD-FYVE protein binds the kinases Akt and PKCζ/α and regulates glucose uptake in adipocytes (submitted).
Ress, A., **Moelling, K.** Interaction partners of the PDZ domain of Erbin (submitted).
Matskevich, A.A., Ziogas, A., Heinrich, J., Quast, S.A. and **Moelling, K.** Short double-stranded oligodeoxynucleotide induces reverse transcriptase-mediated cleavage of HIV RNA and abrogates infectivity of virions (submitted).
Elzaouk, L., Pavlovic, J., **Moelling, K.** Anti-tumor activity of mesenchymal stem cells producing murine IL-12 in a mouse melanoma model (sumitted).
Elzaouk, L., Pavlovic, J., **Moelling, K.** Synergistic anti-tumor activity by vaccination with IL-12-encoding plasmid DNA in combination with the chemokine CCL21 in vivo (submitted).
Lorger, M., **Moelling, K.** Regulation of epithelial wound closure and cell-cell adhesion by interaction of AF6 with actin cytoskeleton (submitted).
Mangesh, J., Vargas, C., **Moelling, K.,** Boisguerin, P., Diehl, A., Krause, G., Schmieder, P., Hagen, V., Schade, M., Oschkinat, H. Making protein-protein interactions drugable: Discovery and 3D structure of low-molecular-weight ligands complexed with the AF6PDZ domain (submitted).
Ress, A., **Moelling, K.** Bcr interferes with β-catenin-Tcf1 interaction (submitted).
Ress, A., Radziwill, G., **Moelling, K.** The PDZ-protein Erbin modulates β-catenin-dependent transcription (to be submitted).
Fritzius, Th., Frey, A.D., Schweneker, M., Mayer, D. and **Moelling, K.** A WD protein binds VAMP2 and PKCζ and increases PKCζ-dependent phosphorylation of VAMP2 (submitted).
Fritzius, Th., Heinrich, J. and **Moelling, K.** WD-FYFE protein regulates differentiation of pre-adipocytes and glucose uptake in adipocytes (to be submitted).

Books

- (Bitte Publikationsliste nach folgenden Arten gruppieren)
  - *Wissenschaftliche Monografien*
  - *Dissertationen*
  - *Habilitationen*

- *Herausgeberschaften (von Einzelwerken)*

Editor of Book on "Viral DNA Vaccines" in „Intervirology“, S. Karger AG, Basel, 43, 4-6, 2000.

- *Eigene Buchbeiträge in Sammelbänden*

"Harrisons Innere Medizin 1,2", 13. Auflage, Deutsche Ausgabe, Blackwell Wissenschafts-Verlag, Berlin (1995) (2002).

Franke D., Pavlovic J., Utesch T.S., von Kleist M., Schultz J., Dollenmaier G., **Moelling K.** "Update on Antiviral DNA Vaccine Research (2000-2003)" in: "Novel Vaccination Strategies", ed. S.H.E. Kaufmann, Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, S. 289-317 (2004).

„Springer Lexikon Medizin“, P. Reuter (Hsg.), Springer-Verlag Berlin, Heidelberg, New York, S. 2185-2197 (2004).

- *Lehrbücher*
- *Kongress-, Tagungs- und Workshopbände (Proceedings)*
  - *Herausgeberschaft*
  - *Eigene Beiträge*

➢ Weitere mögliche Publikationsarten :

- *Wissenschaftliche Editionen (Quellenedition mit Kommentar)*
- *Beiträge zu Loseblattwerken*
- *Werkausgaben, Museumskataloge, Ausstellungskataloge*
- *Skripte, Schulungs-/Trainingsmaterialien*
- *Testmanuale*
- *Wörterbücher, Glossare, Lexika*
- *Übersetzung eines wissenschaftlichen Werkes*
- *Neuauflage von Büchern*
- *Sonstige Buchveröffentlichungen*
  z. B. Bibliographien, Sachbücher, Schulbücher
- *(Mit-)Herausgeberschaften*
  z. B. Zeitschriften/Buchreihen/Newsletter
- *Newsletter-Publikationen*
- *Patentschriften*
- *Audiovisuelle Materialien oder Multimediaprodukte*
- *Beiträge in Tages- und Wochenzeitungen*
- *Graue Literatur*
  z. B. Reports, Forschungsberichte für Drittmittelgeber/Auftraggeber, unveröffentlichte Gutachten, Manuskripte für Rundfunk-, Fernsehsendungen
- *Sonstiges*
  z. B. Internet-Dokumente, Normen, Offenlegungsschriften, Musikalien/Noten, Filme/Videos

11.9.4 National and International Awards and Honors 2000 – 2004

11.9.5 Offers of a Professorship at Other Institutions 2000 – 2004

11.10 Teaching

11.10.1 List of the Diploma Theses That You Supervised

➢ Nach Abschlussjahr geordnet; mit Angabe von Titel, Jahr und Autorin / Autor.

Gesine Rudolph, Diplomarbeit (2000)
Suche nach Interaktionspartnern des EphrinB2 und *in vitro* Angiogenese
Ralph Kretzschmar, Diplomarbeit (2000)
Charakterisierung von AIP, eines neuen mit der Kinase Akt interagierenden Proteins
Karen Schad, Diplomarbeit (2000)

Regulation of the Crosstalk between the PI3K-Akt and the Ras-Raf-MEK-ERK pathway in MCF-7 cells
Sandra Wechsler, Diplomarbeit (2000)
Interaktion des PDZ Proteins AF-6 mit dem Transkriptionsfaktor MyT1
Dag Schauwienold, Diplomarbeit (2000)
Charakterisierung von Bindungspartnern des Raf-1 assoziierten Proteins CNK
Nadine Hardel, Diplomarbeit (2001)
Das PDZ-Protein ERBIN bildet einen Komplex mit der Proteinkinase Bcr und dem Protoonkogenprodukt β-Catenin
Buschmann Catharina, Diplomarbeit (2001)
Untersuchung der Wechselwirkung von PDZ-Domänen mit Liganden
Nina Raetzel, Diplomarbeit (2002)
Gene silencing bei HIV durch Oligonukleotide
Sebastian Zurbriggen, Diplomarbeit (2003)
Influence of HIV-1 specific triplex forming oligonucleotides (TFO) on the RNase H and reverse transcriptase activity *in vitro*.

11.11 Next Generation of Academics/Scientists

11.11.1 List of Completed Dissertations and Habilitations

- chronologisch geordnet; mit Angabe von Titel und Autorin / Autor

Algirdas Ziogas, Doktorarbeit (2001)
Characterization of the multistep activation of Ser/Thr kinase Raf-1 involving phosphorylation and protein protein interactions
Elisabeth Operschall, Doktorarbeit (2001)
DNA Vaccines Encoding Viral Proteins and Strategies to Improve Immune Responses
Ylva Lindman, Doktorarbeit (2001)
Inhibition of tumor formation using the soluble extracellular domain of the EphB3 receptor
Marc Schweneker, Doktorarbeit (2003)
Identification and characterization of two novel proteins interacting with the chemokine- and HIV-1 co-receptor CCR5
Magnus Bosse, Doktorarbeit (2004)
Raf und Akt in Transformation und Differenzierung
Rüdiger Erdmann, Doktorarbeit (2004)
Regulation der Raf Kinase durch Bcr Kinase und Stützprotein CNK
Angelika Reß, Doktorarbeit (2004)
Proteininteraktionspartner von beta-Catenin

11.12 Further Aspects

- Visiting Professors: Gastprofessuren von Angehörigen der Forschungsgruppe 1998-2002 an anderen Institutionen (minimale Dauer 2 Wochen)
    - Editorships: Chief Editor (journals/series); Member of Editorial Boards / Associate Editor (journals/series)
    - Offices / Functions Held at the University (self-administration) and outside
    - Relationships to Government (Politics), the Economy, and Society

11.13 Curriculum Vitae

- CV mit Stationen des akademischen / beruflichen Werdegangs (ohne Auflistung der Publikationen).

Prof. Dr. Karin Mölling

Name:	**Karin Moelling,** born on April 7, 1943 in Meldorf/Dithmarschen (FRG)
1962-1965	**Student** of Physics and Mathematics at the Christian Albrechts University, Kiel
1965	**1st Degree** (Vordiplom)
1966-1968	**Student** of Physics and Mathematics at the Universities of Göttingen and Kiel
1968	**Diploma Degree** in Nuclear Physics at the Institute of Pure and Applied Nuclear Physics, Kiel with Prof. Dr. E. Bagge und Prof. Dr. J. Trümper. **Thesis:** "ANALYSIS OF NON-ISOTROPIC DISTRIBUTION OF COSMIC RAYS"
1968-1969	**Student** at the University of California, Berkeley, USA, with a scholarship award from the *"Studienstiftung des Deutschen Volkes".* Biochemistry and Molecular Biology at the Molecular Biology and Virus Laboratory with Dr. J.M. Chamberlin
1969-1972	**Ph.D. Thesis:** "REPLICATION MECHANISM OF RETROVIRUSES" at the Max-Planck-Institute of Virus Research, Tübingen with Prof. Dr.W. Schäfer and Prof. Dr. H. Bauer. "ANALYSIS OFRETROVIRAL REVERSE TRANSCRIPTASE AND IDENTIFICATION OF RNASE H ACTIVITY"
1972	**Ph.D.** in Nuclear Physics at the Department of Physics at the Eberhard Karls University, Tübingen
1972-1975	**Post-doctoral Fellow** at the Robert-Koch-Institute, Berlin, with Prof. Dr. H. Bauer. Analysis of reverse transcriptase and RNase H
1975-1976	**Employee** at the Institute of Virology at the Justus Liebig University, Giessen with Prof. Dr. H. Bauer. Analysis of viral glycoproteins and precursor-processing
1977	**Habilitation** in the Department of Biophysics, University of Giessen on "REPLICATION OF RETROVIRUSES"
1976-1981	**Head of an Independent Research Group** at the Max-Planck-Institut für Molekulare Genetik, Berlin. Analysis of replication and transformation by retroviruses
1981-1983	**Fellow of the "Heisenberg Fellowship Foundation"** at the Max-Planck-Institut für Molekulare Genetik, Berlin in Department Prof. Dr. H. Schuster. Analysis of molecular mechanisms of malignant transformation by oncogenes
1987	**Apl Professor**, Freie Universität Berlin, Faculty of Medicine, Basic Sciences
1983-1993	**Tenured C3 Professorship** position at the Max-Planck-Institut für Molekulare Genetik, Berlin in Department Prof. Dr. H. Schuster. Oncogenes and proto-oncogenes, regulation of gene expression, tumor markers, replication of HIV
1992-1993 Inc., USA,	Joint appointment as **Director of Cellular and Molecular Biology**, Apollon, resulting in a phase I clinical trial for an HIV DNA vaccine in Zürich
1993-present	**Full Professor** and **Director** of the Institute of Medical Virology, University of Zürich
1996-present	**External Faculty Member** of the Dept. of Med., Free University, Berlin
2005	**Honorary Professorship**, Charité, Humboldt University, Berlin

MEMBERSHIPS

1988	Endowed lecture "Bertha Benz" on "Retroviruses in Cancer and AIDS Research".
1989-1992	Member of Editorial Board of "Archives of Virology".
1992-1994	Member of Editorial Board of "DNA and Cell Biology".

1993	Member of EMBO.
1996	Member of the Technology Council for Technology Transfer and Bio-technology of the German Chancellor, Dr. Helmut Kohl.
1999-present	Member of the Technology and Innovation Council of the City of Berlin

AWARDS

1981	Vincenz **Czerny** Prize for Oncology 1981 for Thomas Bunte und Karin Moelling
1982	Walther und Christiane **Richtzenhain** Prize 1982 "Mechanisms of malignant transformation"
1983	Membership of the European Molecular Biology Organisation (EMBO)
1986	Wilhelm und Maria **Meyenburg** Prize 1986 for scientific contributions to the field of "Carcinogenesis"
1987	**Aronson** Prize for scientific contributions to "Cancer and AIDS Research"
1992	Heinz **Ansmann** Prize for AIDS Research
2005	Honorary Professorship, Charité, Humboldt University, Berlin

PUBLICATIONS

207 Publications

4 Books, 8 Book Chapters

12 PD Dr. J. Pavlovic

12.1 Research 2000-2004

12.1.1 Research Projects

The Antiviral Function of the Interferon-Induced Mx Proteins

The interferon system provides vertebrates with a powerful tool to defend themselves against infecting viruses. Interferons mediate their antiviral activity by inducing the synthesis of about 50 proteins. A few of the interferon-induced proteins such as Mx proteins are shown to function as intracellular mediators of virus resistance. Mx proteins are GTP-binding proteins and have intrinsic GTPase activity.

The overall goal of this project is to elucidate the molecular mechanism of action of Mx proteins. This is achieved by: (a) assessing the antiviral spectrum of Mx proteins in cell culture and in vivo, (b) defining the functional domains of Mx proteins, (c) characterizing the viral target of Mx action and (d) identifying auxiliary factors necessary for Mx activity.

The GTPase and antiviral activity of Mx proteins is controlled by a carboxyterminal GTPase effector domain

The interferon-α/β inducible Mx proteins belong to the functionally diverse dynamin family of large GTPases. Characteristic features are a highly conserved GTP-binding region, an intrinsic GTPase activity and their capacity to form oligomers. The human MxA protein exerts antiviral activity by inhibiting the replication of certain RNA viruses in a GTP-dependent fashion. We have recently demonstrated that backfolding of the C-terminal end of human MxA protein onto a more proximal part of the molecule is a prerequisite for oligomerization. The backfolding of MxA is stabilized by an amphipathic helix LZ1. Prevention of backfolding by mutation of LZ1 at the amino acid position 612 (L612K) leads to the loss of GTPase activity and capacity to oligomerize, but the antiviral activity is retained. We show here that similarly to dynamin, the intrinsic GTPase activity of Mx proteins is mediated by a GTPase effector domain (GED), located at the C-terminus. Mutations in the GED domain (R640A or A653K) abrogated the GTPase activity and the antiviral activity of MxA. Nevertheless, these mutants retain their capacity to form oligomers. Moreover, we show that the highly cytotoxic, monomeric form of MxA [MxA(L612K)] exerts its antiviral activity in the absence of a functional GED or GTP-binding site. Based on these results we propose a model where Mx proteins are synthesized in response to interferon-α/β to form a pre-activated oligomeric structure. In the presence of viral components, MxA may then convert in a GTP-dependent manner to a monomeric, activated form. Once this step is completed MxA no longer requires GTP for its antiviral activity.

Inhibition of Semliki Forest Virus by the Interferon System

Type I Interferons (IFNs) are secreted cytokines that represent an important component of the innate immune system. The induction of many genes by IFN leads to the generation of the so-called antiviral state in the cell. During evolution, viruses have developed various mechanisms to counteract the antiviral activity of the IFN system. Cells that lack a functional IFN system become very sensitive to normally harmless viruses demonstrating the importance of the IFN system in antiviral protection. So far only three IFN-induced antiviral pathways are well described and there is evidence for the existence of additional IFN-mediated defense mechanisms.

We are investigating two closely related Semliki forest viruses (SFV) with different sensitivities

to IFN. The virulent V45 strain is lethal for wild type (wt) mice whereas infection of mice with the avirulent V42 strain remains asymptomatic. Upon IFN treatment *in vitro*, MEFs derived from wt mice are completely protected from the avirulent V42 strain while IFN-stimulated cells are killed by the virulent V45 strain. We were interested in defining the viral sequence determining the IFN sensitivity of SFV.

We have sequenced both virus strains and constructed recombinant cDNA clones derived from both strains. Several differences were detected in the coding region of the non-structural and structural proteins but also in the non-translated region (NTR). Unexpectedly, we discovered differences in the 5' NTR compared with the known published SFV 5' NTR. The avirulent V42 strain contains an additional sequence of 20 nucleotides (nts) at the extreme 5' end, which are lacking in the IFN-resistant virus. These additional nts are predicted to form a hairpin structure preceding the highly structured 5' NTR of SFV. Generation of chimeric viruses containing swapped segments revealed that the additional 20 nucleotides are sufficient to confer IFN sensitivity. Therefore, we termed this sequence Interferon Sensitivity Determining Element (ISDE).

The avirulent ISDE-containing virus is supposed to be closely related to the original isolate whereas the IFN-resistant virus has been derived from the original isolate by passaging in mouse brains. This raises the hypothesis that the ISDE has been maintained in nature but was then rapidly lost upon passaging in mouse brains and/or tissue culture. The virulent IFN-resistant virus therefore represents a mouse-adapted strain. Nevertheless, the question remains why the ISDE is maintained in nature.

Since SFV is a virus that alternatively infects invertebrate and vertebrate hosts, we were interested to investigate the replication of the various generated virus clones in mosquito cells. All virus clones grow equally well in vertebrate cells in the absence of a functional IFN-system or exogenously added IFN. In mosquito cells however, significant differences in the growth kinetics were observed depending on the presence of the ISDE. Although clones containing an ISDE grew with a slower rate during the first days of infection, they reached higher peak titers than the clones lacking the ISDE. Moreover, the presence of the ISDE permitted a sustained production of high virus titer over several days. At 5 days post infection up to 4 log higher virus titer was observed for clones containing the ISDE.

DNA Vaccines against viral infection and cancer
Influenza virus

Nature of the protective immune response elicited by viral antigens encoded by plasmid DNa

DNA vaccines have proven to be effective against many viral infections in animal models. Since the mechanism of protection is not fully understood we wanted to investigate which part of the immune system is responsible by using genetically modified mice and influenza A DNA vaccine as model.

Mouse strains with specific defects in the cellular (perforin-deficient mice) or humoral response (IgM-deficient mice) were analysed. Wild type (C57BL/6), perforin-deficient and IgM-deficient mice were vaccinated with expression vectors encoding the viral envelope protein haemagglutinin (HA), the internal viral nucleocapsid protein (NP) or empty vector as control and subjected to challenge with a lethal dose of influenza A virus homologous to the vaccine DNA. Vaccination with the HA-encoding DNA led to protection of wild type and perforin-deficient animals against influenza virus infection, while IgM-deficient mice succumbed. By contrast, protection of mice vaccinated with NP-encoding DNA was strongly dependent on the dose of challenge virus and was only observed at low doses of virus inoculum but not at higher doses, despite the presence of a specific cytotoxic lymphocyte (CTL) response against NP. Mice vaccinated with HA- or NP-encoding DNA produced specific antibodies against the

corresponding viral protein. These findings show that vaccination with DNA encoding HA elicits a more potent protective immune response against influenza virus infection than DNA encoding NP. The observed protection induced by HA-encoding DNA appears to be predominantly mediated by neutralizing antibodies.

Enhancement of the immune response to viral antigens encoded by plasmid DNA

Professional antigen-presenting cells, in particular dendritic cells (DCs) play a pivotal role in induction of protective immune responses by capturing processing and presenting antigens on MHC molecules to effector or helper T cells. A major drawback of DNA vaccines is their limited efficacy to target antigens to DCs by cross priming. To circumvent this limitation, we evaluated a novel strategy for DNA vaccination, employing a well-established mouse influenza virus model, by directly targeting plasmid encoded viral antigens to DCs. For this purpose plasmids were constructed that encoded the extracellular portion of haemagglutinin (sHA) or the entire coding region of the nucleoprotein (NP) fused to the Fc portion of mouse IgG2a. Immunization of mice with plasmids encoding the membrane bound form of HA or the secreted sHAFc led to a pronounced protective immunity (86 % survival) when challenged with a lethal dose of influenza A virus. Vaccination with DNA encoding NP or sNPFc was much less effective (33 % and 0 % survival, respectively). Vaccination with DNA encoding the membrane bound form of HA induced high titers of neutralizing antibodies and the resulting protective immunity was not affected by *in vivo* depletion of $CD4^+$ or $CD8^+$ T lymphocytes. Remarkably, despite a pronounced protective immunity, vaccination with DNA encoding sHAFc did not yield significant titers of neutralizing antibodies. Moreover, the protective immunity induced by vaccination with DNA encoding sHAFc and the dramatic increase of neutralizing antibodies observed after viral challenge was strongly dependent on the presence of $CD4^+$ T cells. Moreover, this vaccination strategy resulted in a long lasting protective immunity. Taken together, these data indicate that immunization with DNA encoding a membrane bound antigen induced protective immunity by activating Plasma B cells producing high titers of neutralizing antibodies, while immunization with DNA encoding sHAFc targeted to DCs resulted in the activation of memory B cells that produced neutralizing antibodies only after restimulation after viral challenge.

Severe acute respiratory syndrome (SARS)

In November 2002, a new form of infectious pneumonia, known as severe acute respiratory syndrome (SARS), emerged in China. The virus causing SARS belongs to the family of Coronavirus and displays a large, positive stranded RNA genome of more than 29kb. In order to develop a potential vaccine against SARS-CoV, we examine a naked DNA immunization. Using viral genomic RNA as template, we have cloned the entire coding region of the Spike (S) glycoprotein by RT-PCR. Sequence analysis proved sequence identity with the Frankfurt 1 isolate and the S protein was cloned into a mammalian expression vector pVR1012. Correct expression of the protein was verified in mammalian cells by western blot analysis.

Different constructs encoding the S protein were generated in order to improve the immune response to this weak antigen. The modified construct carries a GPI-anchor sequence at the C-terminus of the S protein, missing the transmembrane and cytoplasmic region. GPI-anchor proteins are characterized to arrange in lipid rafts, which we can show with lipid raft extraction. Several features of GPI-anchored proteins have been described to optimize the presentation to immune responsive cells. Furthermore, we substituted the wt signal sequence of the S protein with the haemagglutinin leader (HA) of influenza. The HA leader sequence is expected to transport the protein more efficiently to the cell surface. This design may be useful for other DNA vaccines. The constructs were injected three times as naked DNA. ELISA and immunofluorescence showed diverse specific interaction for all constructs. Neutralization test proved the existence of neutralizing antibodies in mice.

Immunotherapy against breast cancer using Mucin-1 as tumor antigen

Prime-Boost strategy

This project is persued as an EU network grant of the 5th framework (granting period December 1, 2003 to February 28, 2006)

Collaborative partners are Joyce Taylor-Papadimitriou (Coordinator, Cancer Research UK, London, United Kingdom), Thomas Noll (Forschungszentrum Juelich, Germany), Gunnar Hannson, Thommy Nilsson (University of Göteborg, Sweden), Jan Kihlberg (Umea University, Sweden), Henrik Clausen (University of Copenhagen, Denmark), Marianna Nuti (University of Rome, Italy), and David Snary (ICRT, London, United Kingdom)

The aim of this project is to develop a prime boost strategy based on MUC1 antigens for immunotherapy of cancer, where the priming injections will use MUC1 cDNA, and be boosted by a MUC1 glycoprotein corresponding to a cancer associated glycoform. Selection of the most effective glycoform will allow us to design a prime boost protocol for a future Clinical Trial. Further preclinical studies will continue to 1. Modify cDNA constructs for improved antigen presentation. 2. Further define cancer associated glycosylation patterns.

The epithelial membrane mucin MUC1 is over expressed and aberrantly glycosylated in more than 90 % of Breast, Ovarian and Pancreatic Cancers as well as in a proportion of other carcinomas. Both humoral and cellular responses to MUC1 have been found in breast and ovarian cancer patients Small trials with MUC1 based cancer vaccines are in progress in Europe and North America.

MUC1 sequences contain classical HLA Class I epitopes which flank a domain made of tandem repeats, each of which contains 5 potential glycosylation sites

The project involves: Production of the following materials (i) Tumour-associated glycoforms of MUC1 produced in CHO cells modified by transfection of specific glycosyl transferases (ii) MUC1 glycopeptides prepared by chemo-enzymatic synthesis (iii) MUC1 cDNAs.

Some of the participants have collaborated to produce a secreted MUC1 glycoprotein from wild type (WT) CHO cells. The dominant O-glycan is SialylT, (ST) and a MUC1 product carrying mainly T O-glycans can be generated with sialidase. The number of sites glycosylated is variable between repeats but some are fully glycosylated. These products MUC1-T(wt) and MUC1-ST(wt) are now under evaluation by us in mouse models. However, some breast cancer cell lines have all five sites (5s) glycosylated on each tandem repeat. Moreover, we have recently found that a MUC1 glycopeptide carrying 5 STn O-glycans per repeat can overcome humoral tolerance in transgenic mice expressing human MUC1.

Dendritic cells for immuno therapy of breast cancer

This project is persued as an EU network grant of the 5th framework (granting period November 1, 2003 to October 31, 2005).

Collaborating partners are: Thomas Noll (Coordinator, Forschungszentrum Juelich, Juelich, Germany) Joyce Taylor-Papadimitrou (ICRF, London, United Kingdom),), Jacques Bartholeyns (IDM, Paris, France) Yvette van Kooyk (University of Amsterdam, Amsterdam, The Netherlands), Gerard Bos (University of Maastricht, Maastricht, The Netherlands), Henrik Clausen (University of Copenhagen, Copenhagen, Denmark), Gunnar Hannson (University of Göteborg, Göteborg, Sweden),

Dendritic cells (DC) are the most potent antigen-presenting cells for the initiation of antigen-specific immune responses. In addition to their ability to efficiently acquire and process

antigens, DC express high levels of MHC class I and class II molecules as well as costimulatory molecules essential in antigen presentation. Therefore dendritic cells are capable of recruiting the multiple components of the immune system.

Hence, numerous methods of delivery of tumor antigens to dendritic cells, as well as routes and schedules of administration to cancer patients, have been developed. The first dendritic cell clinical studies have indicated this form of vaccination as feasible and safe; furthermore, in some cases, objective clinical responses were observed. These encouraging preliminary data require further extensive investigations, which should address the technical and biological problems of generating and loading of human dendritic cells.

This project aims for the development of an effective immunotherapy for breast cancer, based on dendritic cell vaccines.

This includes the generation of dendritic cells, the production of breast cancer related immunogens, the development of new and the improvement of existing strategies for enhanced antigen presentation by DC's, the *in vitro* evaluation of immune response using patients T-cells, the *in vivo* evaluation of tumor rejection in mouse models and the transfer of the most successful approach to GMP as well the design of a clinical trial.

Our part of the project is to generate retroviral vectors for efficient transduction of DCs. Transfection of dendritic cells encoding tumor antigen have previously been shown to induce a T helper cell response recognising the antigen of interest. To evaluate the suitability of this approach for breast cancer we have produced a lentiviral vector expressing MUC1. With this viral construct human DCs were successfully transduced and expressed MUC1 at the cell surface. We currently test whether MUC1-specific peptides are presented by MHC class 1 molecules.

Experimental approaches for Immunotherapy with plasmid DNA encoding cytokines

IL-12 in combination with IP -10

IP-10 has been shown to display antitumor and antimetastatic properties by immunological as well as antiangiogenic mechanisms when applied by genetically engineered tumor cells, as recombinant defective adenovirus or as recombinant protein. Its immunological properties appear to be based on the attraction of monocytes, T lymphocytes, modestly neutrophils and possibly NK cells whereas its antiangiogenic effect may rely on the suppression of both endothelial cell proliferation and differentiation of endothelial cells into tubular capillary structures. For this reason, we investigated antitumor and antimetastatic efficiency of IP-10-encoding DNA, which was further enhanced by coadministration of IL-12 DNA in a melanoma lung carcinoma model. Plasmid DNA encoding IP-10 substantially reduced the establishment of metastases when injected systemically by the intramuscular route. In contrast to the primary tumor model, the antimetastatic effect of DNA encoding IP-10 was primarily mediated by NK cells. Compared to DNA encoding IL-12, therapy with DNA encoding IP-10 exhibits lower efficacy against primary melanoma tumors but equivalent efficacy against primary Lewis lung tumors and against B16F10 lung metastases formation. Co-administration of DNA encoding IP-10 and IL-12 enhanced the anti-tumor activity of IL-12 in the lung-metastasis model but had little effect in the local treatment of established subcutaneous tumors even though a substantial increase of $CD4^+$ T cell infiltration was observed.

IL-12 in combination with CCL21

The aim of this of this study was to develop an effective antitumor vaccine strategy by combining the benefit of IL-12 DNA and chemokines, such CCL21 and CCL19, *in vivo* to produce an efficient immunotherapy for malignant melanoma. It is well established that chemokines and their receptors are the key elements that direct lymphocytes and antigen-presenting cells (APCs) to different areas in secondary lymphoid organs. Recently Sanchez-Sanchez et al. were able to show that CCL21 and CCL19 which play a role in chemotaxis induce antiapoptotic signaling in mature dentritic cells (DCs) via stimulation of their receptor CCR7 (Sanchez-Sanchez et al, 2004). Since CCL21 and CCL19 are powerful attractants that direct DCs, we expect that once injected peritumorally, they will exert their effect as chemoattractants and mobilize the DCs resulting in increase of the antitumor effect of IL-12 in a combined treatment. DCs play a major role in the initiation of the immune response. For this purpose, we assessed the therapeutic effect of IL-12 DNA in combination with these chemokines (CCL21 and CCL19) in a primary tumor model. The data produced so far clearly indicate that combination therapy with IL-12 applied as DNA encoding IL-12 and the chemokine CCL21 either applied as recombinant protein or as DNA encoding CCL21 has a synergistic antitumoral effect in the mouse tumor model.

12.1.2 Research Cooperation Agreements, Partnerships

Prof. O. Haller, Institut für Medizinische Mikrobiologie und Hygiene, Abteilung Virologie, Universität Freiburg, Deutschland. (Antiviral function of Mx proteins).

Prof. S. Stäheli Institut für Medizinische Mikrobiologie und Hygiene, Abteilung Virologie, Universität Freiburg, Deutschland (Antiviral function of Mx proteins, regulation of expression of Mx proteins)).

Prof Martin Billeter, Institut für Molekularbiologie, Abteilung I, Universität Zürich (Virus cell interaction of measles virus).

Prof. H. Feldmann, Institut fur Virologie, Philipps-Universitat, Marburg, Deutschland (in vivo model for Hantaan virus).

Prof. Francoise Stoll-Keller, Universite Louis Pasteur Strasbourg, France (Semliki Forest virus replicons as expression vectors).

Prof Dr. R. Dummer, Universitätsspital, Klinik für Dermatologie, Universitätsspital Zurich.

Prof. R. Cattaneo, Mayo Clinic, Rochester Minnesota, USA (Virus cell interaction of measles virus, in vivo model for measles virus).

12.1.3 Expenditure of third party funds

Co-Applicant

Schweizerische Krebsliga SKL 983-02-2000): Dysfunctional interferon signaling in lymphoma: molecular analysis. (co-application with R. Dummer). 1.8.2000-30.7.2002.

Schweizerische Krebsliga SKL (01217-02-2002): Dysfunctional interferon signaling in lymphoma: molecular analysis. (co-application with R. Dummer) 1.8.2002-30.1.2005.

5. framework EU Network Grant: (QLK3-2002-01980) Development of a an immunotherapy for breast cancer-based on dendritic cells. (co-application with K. Moelling) 1.11.2002-30.10.2005.

5. framework EU Network Grant: (QLK3-2002-02010) A prime boost strategy for immunotherapy of breast and ovarian cancer. (co-application with K. Moelling) 1.12.2002-30.11.2005.

12.1.4 Publications, 2000-2004

Articles in Academic/Scientific Journals

Peer reviewed articles

Mrkic, B., Odermatt, B., Klein. M.A., Billeter, M.A., **Pavlovic, J.**, and Cattaneo, R. (2000) Lymphatic dissemination and comparative pathology of recombinant measles viruses in genetically modified mice. *J. Virol*. **74:**1364-1372.

Nawrath, M., **Pavlovic, J.**, and Moelling, K. (2000) Inhibition of human hematopoietic tumor formation by targeting a repressor Myb-KRAB to DNA. *Cancer Gene Ther*. **6**:963-72.

Heinicke, T., Radziwill, G., Nawrath, M., Rommel, C., **Pavlovic, J.**, Moelling, K. (2000) Retroviral gene transfer of dominant negative raf-1 mutants suppresses ha-ras-induced transformation and delays tumor formation. *Cancer Gene Ther*. **5:**697-706.

Schultz, J., **Pavlovic, J.**, Moelling, K. (2000) Immune modulation in cancer using DNA inoculation--antitumour effect of interleukin-12. *Dev. Biol.* **104:**109-114.

Schultz, J., Heinzerling, L., **Pavlovic, J.**, Moelling, K . (2000) Induction of long-lasting cytokine effect by injection of IL-12 encoding plasmid DNA. *Cancer Gene. Ther.* **7:**1557-1565.

Dummer, R., Dobbeling, U., Geertsen, R., Willers, J., Burg, G., and **Pavlovic, J.** (2001) Interferon resistance of cutaneous T-cell lymphoma-derived clonal T-helper 2 cells allows selective viral replication. *Blood* **97:**523-527.

Bouloy, M., Janzen, C., Vialat, P., Khun, H., **Pavlovic, J.**, Huerre, M., Haller O. (2001) Genetic evidence for an interferon-antagonistic function of rift valley fever virus nonstructural protein NSs. *J. Virol.* **75:**1371-1377.

Nawrath, M., **Pavlovic, J.**, Moelling, K. (2001) Synergistic effect of a combined DNA and peptide vaccine against gp100 in a malignant melanoma mouse model. *J. Mol. Med*. **79:**133-142.

Roscic-Mrkic, B., Schwendener, R.A., Odermatt, B., Zuniga, A., **Pavlovic, J.**, Billeter, M.A., Cattaneo, R. (2001) Roles of macrophages in measles virus infection of genetically modified mice. *J Virol* **75:**3343-3351.

Heinzerling, L., Dummer, R., **Pavlovic, J**., Schultz, J., Burg, G., Moelling, K. (2002) Tumor regression of human and murine melanoma after intratumoral injection of IL-12-encoding plasmid DNA in mice. *Exp. Dermatol.* **11:**232-240.

Keyser, J., Schultz, J., Ladell, K., Elzaouk, L., Heinzerling, L., **Pavlovic, J.**, Moelling, K. (2004) IP-10-encoding plasmid DNA therapy exhibits anti-tumor and anti-metastatic efficiency. *Exp Dermatol.* **13:**380-390.

Wichmann, D., Grone, H.J., Frese, M., **Pavlovic, J**., Anheier, B., Haller, O., Klenk, H.D., Feldmann, H. Hantaan virus infection causes an acute neurological disease that is fatal in adult laboratory mice (2002) *J Virol*. **76**:8890-8899.

Schultz, J.G., Salzer, U., Mohajeri M.H., Franke, D., Heinrich, J., **Pavlovic, J.,** Wollmer, M.A., Nitsch, R.M., Moelling, K. Antibodies from a DNA peptide vaccination decrease the brain amyliod burden in a mouse model of Alzheimer's disease. (2004) *J Mol Med.* **82:**706-714.

Jilek, S., Zurkaulen. H., **Pavlovic, J.,** Merkle, H.P., Walter, E. Transfection of a mouse dendritic cell line by plasmid DNA-loaded PLGA microparticles in vitro. (2004) *Eur J Pharm Biopharm* 58:491-499.

Books

Kongress, Tagungs-und Workshopbände

Operschall, E., **Pavlovic, J.**, Nawrath, M., Molling, K. (2000) Mechanism of protection against influenza A virus by DNA vaccine encoding the haemagglutinin gene. *Intervirology* **43:**322-330.

Pavlovic, J., Schultz, J., Hefti, H.P., Schuh, T., Molling, K. (2000) DNA vaccination against La Crosse virus. *Intervirology* **43:**312-321.

12.2 Teaching

12.2.1 List of Diploma theses

2000

Dominik Meier, Universität Zürich: Vergleich der Wechselwirkungen von Polymerasekomplexen aviärer und humaner Influenza-A-Viren mit dem antiviralen MxA Protein.

2002

Nathalie Constantin, ETH Zürich: Identification of the viral target domain of MxA.

2004

Junmin Hu, ETH Zürich: Generation of a replication competent Semliki Forest virus pseudotyped with the glycoprotein of vesicular stomatitis virus.

12.3 Next Generation of Academics/Scientists

12.3.1 List of completed Dissertations and Habilitations

2004

Johanna Keyser Dr. med, Universität Zürich: IP-10-encoding plasmid DNA therapy exhibits anti-tumor and anti-metastatic efficiency.

2005

Stefan Deuber, Dr. rer. nat. ETH Zürich: interference of the IFN-system with viral replication: Identification of a IFN-sensitivity determining element in the genomic RNA of Semliki forest virus.

12.4 Curriculum vitae

Name:	Pavlovic Jovan
Date of Birth:	April 3, 1953
Nationality:	Swiss
Family Status:	Married, 2 children
Address:	Institute of Medical Virology University of Zurich Gloriastrasse 30, CH-8006 Zurich, Switzerland Phone: (044) 634 26 56
	Education
April 1975	Matura type C, Zürich
Oct. 1975 - Nov. 1980	Studies in Biology, University of Zurich
Nov. 1980	Diploma in Botany at the Institute of Plant Biology, University of Zurich
Dec. 1980 - July 1985	Ph.D. thesis at the Institute of Plant Biology, University of Zurich
July 1985	Degree of PhD University of Zurich
Nov. 1995	Habilitation in the Faculty of Medicine, University of Zurich
	Positions
Aug. 1979 - Feb. 1985	Teaching assistant at the Institute of Plant Biology, University of Zurich
June 1985 - July 1987	Postdoctoral fellow with Dr. R.W. Dottin at the Department of

	Biology, Johns Hopkins University, Baltimore, MD
Nov. 1987 - April 1988	Postdoctoral fellow with Dr. R.W. Dottin at the Department of Biological Sciences, Hunter College, CUNY, New York, NY
May 1988 - Sept. 1989	Postdoctoral fellow with Dr. O. Haller at the Institute of Immunology and Virology, University of Zurich
Oct. 1989	Oberassistent at the Institute of Immunology and Virology, University of Zurich
May 1996 - present	Wissenschaftlicher Abteilungsleiter at the Institute of Medical Virology, University of Zurich

13 OA Dr. Gerald Radziwill

13.1 Research 2000 – 2004

13.1.1 Research Projects

- Detailliertere Beschreibung als in 6.1.2

The regulation and the function of the mitogen-activated protein kinase cascade composed of Raf, MEK and ERK is a longstanding interest of this group. Protein phosphorylation events and protein-protein interactions are the main mechanisms how Raf proteins and the Raf signal pathway is regulated. In the last years this group focused on scaffold proteins, which organize signaling networks. Many of these scaffold proteins are PDZ-domain containing proteins. Three of them are analysed in respect of Raf signaling and as integrator of different signal pathways. AF-6 and Erbin are proteins interacting with activated Ras and thereby interfering with the Ras/Raf signaling. The multidomain protein CNK interacts with Raf and the tyrosine kinase Src, the second main activator of Raf apart from Ras, and allows Src-dependent activation of Raf.

Regulation of Raf signaling

Mechanisms of Raf activation

Raf headed the mitogen-activated protein kinase cascade composed of Raf, MEK and ERK. Raf kinase activity is regulated by phosphorylation and interaction with specific inhibitory or activating proteins. The main activators of Raf are the GTPase Ras and the tyrosine kinase Src. We identified the protein 14-3-3 as negative regulator of Raf activation and demonstrated that activated Ras displaced 14-3-3 from its N-terminal binding site on Raf-1 [Rommel et al., 1996][1]. This mechanism of Raf regulation was verified in vivo in *Drosophila melanogaster* [Rommel et al., 1997]. Binding of 14-3-3 to the amino terminus depends on phosphorylation of Ser 259. Small peptides comprising this region prevent activation of Raf-depending and subsequently ERK phosphorylation [Radziwill et al., 1996].

In further studies we identified a positive feedback mechanism, which is mediated by MEK1 and resulted in stimulation of Raf-1 activity independently of Ras and Src [Zimmermann et al., 1997]. Interestingly we were able to demonstrate that activation of Raf-1 in mitotic cells is also independent of Ras and tyrosine phosphorylation of Raf-1 [Ziogas et al., 1998]. This means that under certain conditions Raf-1 activation can occur without the classical Raf-1 activators Ras and Src.

Raf proteins are involved in cellular transformation. We demonstrated that amino terminal parts of Raf-1 can act as dominant negative proteins that inhibits Ras-dependent transformation and reduces tumour formation of ras-transformed cells in nude mice [Heinicke et al., 2000].

Recently we analysed the function of the Ras-binding protein AF-6 (see below). We demonstrated that the kinase Bcr phosphorylates AF-6, which allows binding of Bcr to PDZ domain of AF-6. Phosphorylation induced a conformational change of AF-6 and increased its binding to activated Ras. Thereby Bcr downregulates the Raf/MEK/ERK pathway via AF-6 [Radziwill et al., 2003]. Since Bcr is a kinase constitutively active in quiescent cells this mechanism may be involved in downregulation of the basal Ras activity to keep a cell in a quiescent state.

CNK1, a scaffold protein that regulates Src-mediated Raf-1 activation

[1] [ ] references of G. Radziwill

In the last few years the scaffold proteins attract more and more attention. Scaffold proteins are multidomain proteins that are involved in clustering of receptors, organising of the cytoskeleton and building up platforms for signaling complexes. The present focus concerning Raf signaling is the function of the multidomain protein CNK (connector enhancer of Ksr). CNK has been first described as regulator of the Ras/Raf pathway in *Drosophila*. CNK also interacts with other signal transducers and is involved in the regulation of proliferation, differentiation, apoptosis and organisation of the cytoskeleton. We identified CNK as a scaffold protein in the Raf-1 signaling pathway [Ziogas et al., 2005].

We generated a polyvalent antibody against human CNK1 (Dr. Bootz, Biologisches Zentrallabor Zürich). With this antibody but not with a commercially available one we were able to detect endogenous CNK in several human epithelial and fibroblast cell lines and to coprecipitate Raf-1 and CNK1. Furthermore we showed that CNK forms a trimeric complex with preactivated Raf-1 and its upstream activator Src, a non-receptor tyrosine kinase. CNK1 regulates the activation of Raf-1 by Src in a concentration-dependent manner typical for scaffold proteins. Downregulation of endogenously expressed CNK1 by small inhibitory RNA interferes with Src-dependent phosphorylation and activation of Raf-1. Thus, CNK1 allows a cross-talk between Src and Raf-1 and is essential for full activation of Raf-1 [Ziogas et al., 2005].

The multidomain protein CNK as integrator of signaling pathways

The multidomain protein CNK functions as a scaffold that connects upstream activators and downstream targets of Ras- and Rho-dependent signaling pathways and may allow cross-talks between theses pathways. CNK mediates proliferative as well as antiproliferative responses including differentiation and apoptosis depending on the cellular system.

The scaffold protein CNK1 interacts with the angiotensin II type 2 receptor

The angiotensin II type 2 (AT_2) receptor is an atypical G protein-coupled receptor that negatively cross-talks with receptor tyrosine kinases to inhibit cell proliferation and to induce apoptosis. These antiproliferative effects of the AT_2 receptor are mainly exerted by the activation of protein phosphatases coupled to the inhibition of the Raf/MEK/ERK signal cascade. The AT_2 receptor can also promote neuronal differentiation by activation of the Raf/MEK/ERK pathway and thereby synergizes with the nerve growth factor receptor.

We were able to demonstrate a physical interaction between CNK1 and the AT_2 receptor in an overexpression system as well as in mouse heart tissue. The exchange of a conserved leucine residue in the conserved region in CNK (CRIC) increased the binding affinity of the CNK1 protein to the AT_2 receptor. This amino acid exchange may induce a conformational change that increases the accessibility of the CRIC domain for binding to the AT_2 receptor. An insertion of a negatively charged amino acid stretch into the linker region between the N- and the C-terminal part of CNK1 strengthens the interaction between CNK1 and the AT_2 receptor in a Ras-regulated manner. This indicates that the activation state of a cell modulates the binding between CNK1 and the AT_2 receptor [Fritz and Radziwill, 2005].

What could be the functional significance of the CNK1/AT_2 receptor interaction? First, CNK proteins as well as the AT_2 receptor act in differentiation of neuronal cells mediated by the Rap1/B-Raf/MEK/ERK pathway. Second, CNK and the AT_2 receptor can modulate ERK activity not only in neuronal cells but also in other cell systems. The AT_2 receptor can induce anti-proliferative signaling by stimulating the protein phosphatases SHP-1, MKP-1and PP2A. A complex formed of the AT_2 receptor, CNK and Raf may facilitate dephosphorylation of Raf by PP2A and thereby downregulates mitogenic effects. Third, CNK1 and the AT_2 receptor both can activate caspase-3 to drive cells into apoptosis. Therefore CNK1 may be linked with the AT_2 receptor-induced apoptosis. Thus, CNK may allow the interaction of the AT_2 receptor with its downstream targets and thereby mediates anti-proliferative effects including growth inhibition, differentiation and apoptosis.

Search for interaction partners of CNK proteins

CNK1 is a multidomain protein with different protein-protein interaction domains such as a SAM domain, a CRIC domain, a PDZ domain located at the N-terminus and a Pro-rich region located at the C-terminus. We performed a yeast two-hybrid screen with the N-terminus and C-terminus of CNK1 as bait and a human B-cell or human brain cDNA library as prey. Among the proteins identified as binding partners for CNK1 we will focus on two proteins: the cdc2 like kinase Clk1 and the protein formin homologue overexpressed in spleen FHOS.

Clk1 is a neuronal dual-specificity kinase similar to MEK. Clk-1 phosphorylates substrates in the nucleus, such as Ser/Arg-rich splicing factors, as well as in the cytoplasm such as the phosphatase PTP-1B involved in the insulin pathway. Thus, CNK may act via Clk1 on the insulin pathway and on cell differentiation. We already verified the interaction found in the yeast two-hybrid system by pull-down assays and co-precipitation experiments. In addition we were able to show that Clk1 can phosphorylate CNK1 in vitro. In further experiments we will study the effect of Clk1-dependent phosphorylation on cellular localization of CNK and on a possible effect on the Raf-1 pathway. We will also test whether CNK can influence the activity of Clk1 and thereby is involved in Clk1-dependent regulation of the insulin pathway.

FHOS is a member of the diaphanous-related formin (DRF) proteins. FHOS contains formin homology (FH) regions, a coiled-coil region, a collagen-like domain and two nuclear localisation signals. DRF proteins are involved in development, cell survival, cytokinesis, cell motility and cell polarity. The DRF proteins mDIA1 and mDIA2 couple Rho and Src during signaling and regulation of the actin cytoskeleton organisation. FHOS interacts with Rac1 and induces transcription from the serum response element. MEK inhibitors block FHOS-dependent SRF activation, indicating that the Raf/MEK/ERK pathway may be involved. In addition it has been shown that FHOS enhanced insulin-stimulated glucose uptake in L6 cells probably as connector between glucose transporter containing vesicles and the cytoskeleton. Till now we verified the interaction between CNK1 and FHOS by pull-down and co-precipitation experiments. Moreover we identified FHOS as binding partner and substrate for Src. In further experiments we will test whether CNK affects the FHOS-dependent stimulation of the transcription factor SRF. Activated SRF can be monitored by phosphospecific antibodies. Since activation of SRF involves activation of the Raf/MEK/ERK pathway we will study whether CNK and FHOS modulate Raf-dependent SRF activation.

Interestingly, both Clk1 and FHOS seem to be involved in insulin-dependent events, regulation of the insulin receptor activity and glucose uptake, respectively. Raf-1 also plays a role in the insulin pathway. The Raf/MEK/ERK cascade is activated by insulin and involved in insulin-induced cell differentiation during development. Since several interaction partners of CNK1 mediate insulin-dependent stimulation we will test whether CNK1 participates in this pathway. Therefore, we postulate that CNK1 acts as a platform for proteins mediating the response to insulin stimulation.

Regulation of signaling by PDZ domains

Protein-protein interactions are the main mechanism for building up and for regulation of complex signaling networks. The PDZ domain and PDZ domain containing proteins are a focus of the IMV for some years. PDZ domains are about 80-90 residues in length and recognize short peptide motifs with a hydrophobic amino acid such as valine, leucine or isoleucine at the extreme carboxy terminal end. PDZ proteins are a growing family of proteins involved in different aspects of signal transduction such as receptor clustering, recruitment of cytoplasmic proteins to the plasma membrane and organisation of the cytoskeleton.

Concerning the Raf signaling pathway this group focuses on 3 PDZ domain containing proteins: the Ras-binding proteins AF-6 and Erbin and the Raf-binding protein CNK.

Bcr is a PDZ domain binding kinase that phosphorylates AF-6 and downregulates Ras/Raf signaling

The protein kinase Bcr is a negative regulator of cell proliferation and oncogenic transformation. We demonstrated that Bcr is a ligand for the PDZ domain of AF-6. AF-6 is a Ras-binding protein mainly located at cell-cell junctions. Bcr and AF-6 co-localize in epithelial cells at the plasma membrane. The kinase Bcr is constitutively active in quiescent cells and can phosphorylate AF-6, which subsequently allows efficient binding of Bcr to AF-6. Bcr, AF-6 and Ras form a trimeric complex. Phosphorylation of AF-6 increases its affinity to Ras and a mutant of AF-6 that lacks a specific phosphorylation site for Bcr shows a reduced binding to Ras. Bcr wild type but not Bcr mutants defective in binding to AF-6 interferes with the Ras-dependent stimulation of the Raf/MEK/ERK pathway. In addition, phosphorylation of AF-6 correlates with a decrease in Ras-dependent activation of the Raf/MEK/ERK pathway. Thus, AF-6 functions as a scaffold-like protein that links Bcr and Ras to cellular junctions. This trimeric complex is able to down-regulate Ras-mediated signaling at sites of cell-cell contact to maintain cells in a non-proliferating state [Radziwill et al., 2003].

Identification of proteins interacting with the PDZ domain of Erbin

Erbin was first identified as interaction partner of the receptor tyrosine kinase ErbB2. ErbB2 binds via its C-terminal sequence, which is a typical ligand for PDZ domains, with the PDZ domain of Erbin. In addition Erbin binds via its leucine rich region with activated Ras and thereby interferes with Ras-dependent signaling.

In collaboration with Dr. R. Volkmer-Engert and Dr. P. Boisguerin (Institute of Medical Immunology, Charité, Berlin, Germany) a general assay was chosen to detect ligands for the PDZ domain of ERBIN. A library of 6223 peptides representing the carboxy terminal 11 residues of human proteins of the SWISSPROT database was spotted on a filter and screened with the ERBIN-PDZ domain fused to glutathionine-S-transferase. The intensity of the interactions was quantified by anti-GST antibody coupled to horseradish peroxidase. Among the 20 strongest binder the peptides of beta-Catenin and Bcr appear, whereas the carboxy terminal peptide of ErbB2 was a weak binder [Boisguerin et al., 2004]. This study indicates that Erbin may integrate different signaling pathway by binding via the leucine rich region to Ras and via the PDZ domain to other signal transducers.

ERBIN inhibits beta-Catenin mediated signaling

Beta-Catenin, which we identified as binding partner for the PDZ domain of ERBIN, is complexed with E-Cadherin at cell-cell junctions. In addition beta-Catenin complexed with the T-cell factor TCF regulates gene expression in the Wnt-signaling pathway. This pathway is constitutively activated in several types of human cancer. We demonstrated that Erbin can bind to beta-Catenin and inhibits its function as transcriptional transactivator (A. Ress, G. Radziwill, K. Moelling; manuscript in preparation).

We verified the interaction between beta-Catenin and Erbin found in vitro by co-immunoprecipitation experiments. The interaction between Erbin and beta-Catenin depends on the PDZ domain of Erbin and the C-terminal leucine residue of b-catenin. To prove the interaction between the endogenously expressed proteins we generated an antibody directed against the PDZ domain of ERBIN (Dr. Bootz, Biologisches Zentrallabor der Universität Zürich). This antibody was able to detect endogenous ERBIN in several epithelial cell lines and allows detection of Erbin coprecipitating with beta-Catenin. Beta-Catenin dependent signaling can be analysed by the TOP/FOP reporter assay (Upstate Biotechnology Inc.). Erbin overexpressed in HEK293 cells inhibited the beta-Catenin pathway. Erbin also inhibited the expression of c-Myc a well-characterized target gene of beta-Catenin. Immunofluorescence studies showed that ERBIN overexpressed in HEK 293 is a cytoplasmic protein that is also present at the plasma membrane. Truncation of the leucine-rich repeat results in a protein that locates to the sites of cell-cell contact but also to the nucleus. This PDZ domain containing variant behaved contrary to the wild type protein. It activates beta-Catenin-dependent

signaling. Splice variants of ERBIN RNA have been described but their expression is still unclear. This means that Erbin can function as an activator or an inhibitor of the beta-catenin signal pathway depending of its cellular localisation.

Negative regulation of c-Src by binding to PDZ domain(s)

The tyrosine kinase Src is one of the main activators of Raf-1 but also has many other functions. Recently we demonstrated that the scaffold protein CNK1 binds to Src and Raf-1 and thereby allows Src-dependent phosphorylation and activation of Raf-1 [Ziogas et al., 2005]. In a current project we hypothesize that c-Src is a ligand of PDZ domains, since the C-terminal four amino acids of c-Src display a class III PDZ binding motif. Destruction of this PDZ ligand motif by exchange of the very C-terminal hydrophobic leucine by alanine (Src L529A) increased overall tyrosine phosphorylation of cellular proteins and of the specific Src substrate cortactin compared to c-Src wild type (Src WT). This indicates that c-Src is restricted by interaction of its C-terminus with PDZ domain proteins and that destruction of this interaction by mutating the PDZ binding motif leads to an increase of substrate phosphorylation. In contrast, the mutant Src can phosphorylate many targets in a promiscuous fashion, a property known for v-Src, which does not contain a PDZ ligand motif.

In order to compare wild type Src with the L to A mutant without the background of endogenous c-Src and other ubiquitously expressed Src family members, we used SYF cells, a fibroblast cell-line in which Src, Yes and Fyn are knocked-out so that they cannot supplement each other. These cells were transduced with retroviral vectors to allow inducible expression of Src wild type and the mutant (Dr. Jochen Heinrich, IMV). With these cells we analyzed different biological properties using assays for colony formation, focus formation, the invasivity and cell deadhesion (in cooperation with Dr. Jochen Heinrich and Dr. Andreas Weiss, IMV). These assays confirmed an elevated transformational potential of the c-Src mutant carrying a single amino acid exchange at its C-terminus compared to wild type c-Src.

The major question is what are the natural PDZ domain(s) binding to c-Src. Two different types of yeast-two-hybrid screens failed because the c-Src peptides used as bait activated transcription without interaction partners. As an alternative to identify PDZ domains interacting with c-Src, Dr. Andreas Weiss performed PDZ domain arrays (Panomics, Inc.) and found two PDZ domains interacting with c-Src. In addition we identified c-Src as a ligand of the PDZ domain of AF-6. Overexpression of the PDZ domain interferes with c-Src dependent signaling. This study is still ongoing.

References

Radziwill G, Steinhusen U, Aitken A, Moelling K. Inhibition of Raf/MAPK signaling in Xenopus oocyte extracts by Raf-1-specific peptides. Biochem Biophys Res Commun. 227, 20-6 (1996).

Rommel C, **Radziwill G**, Lovric J, Noeldeke J, Heinicke T, Jones D, Aitken A, Moelling K. Activated Ras displaces 14-3-3 protein from the amino terminus of c-Raf-1. Oncogene 12, 609-19 (1996).

Rommel C, **Radziwill G**, Moelling K, Hafen E. Negative regulation of Raf activity by binding of 14-3-3 to the amino terminus of Raf in vivo. Mech Dev. 64, 95-104 (1997).

Zimmermann S, Rommel C, Ziogas A, Lovric J, Moelling K, **Radziwill G.** MEK1 mediates a positive feedback on Raf-1 activity independently of Ras and Src. Oncogene 15, 1503-11 (1997).

Ziogas A, Lorenz IC, Moelling K, **Radziwill G**. Mitotic Raf-1 is stimulated independently of Ras and is active in the cytoplasm. J Biol Chem. 273, 24108-14 (1998).

Heinicke T, **Radziwill G**, Nawrath M, Rommel C, Pavlovic J, Moelling K. Retroviral gene transfer of dominant negative raf-1 mutants suppresses Ha-ras-induced transformation and delays tumor formation. Cancer Gene Ther. 7, 697-706 (2000).

Radziwill G, Erdmann RA, Margelisch U, Moelling K. The Bcr kinase downregulates Ras signaling by phosphorylating AF-6 and binding to its PDZ domain. Mol Cell Biol. 23, 4663-72 (2003).

Boisguerin P, Leben R, Ay B, **Radziwill G**, Moelling K, Liying D, Volkmer-Engert R. An improved method for the synthesis of cellulose membrane-bound peptides with free C-Termini useful for PDZ domain binding studies. Chemistry & Biology 11, 449-459 (2004).

Ziogas A, Moelling K, **Radziwill G.** CNK1 is a scaffold protein that regulates Src-mediated Raf-1 activation. J. Biol. Chem. 280, 24205-24211 (2005).

Fritz RD., and **Radziwill G.** The scaffold protein CNK1 interacts with the angiotensin II type 2 receptor. Biochem Biophys Res Commun., in press.

13.1.2 Research Cooperation Agreements, Partnerships

Prof. Dr. Heinz Schaller, Zentrum für Molekulare Biologie Heidelberg (ZMBH), Heidelberg, D (HBV: host cell - virus crosstalk; HBV-associated kinase).
Dr. Dietmar Benke, Pharmakologisches Institut, Universität Zürich, CH (yeast two-hybrid screen for Gaba-receptor 2A interacting proteins).

Prof. Dr. Koji Owada, Institute of Molecular and Cellular Biolgy for Pharmaceutical Sciences, Kyoto Pharmaceutical University, Japan (regulation of Src family kinases).

Prof. Dr. Hartmut Oschkinat, Forschungsinstitut für Molekulare Pharmakologie, Berlin, D (PDZ domains).

Dr. Rudolf Volkmer-Engert und Prisca Boisguerin, Institut für Medizinische Immunologie, Charité-Universitätsmedizin Berlin, D (PDZ domains).

Dr. H Funke-Kaiser and Prof. Dr. Thomas Unger, Institut für Pharmakologie und Toxikologie, Charite-Universitätsmedizin, Berlin, D (interaction between angiotensin receptor type 2 and CNK).

Dr. A. Haake and Dr. A. Neild, Center of Mechanics, Swiss Fereral Institute of Technology, Zurich , CH (positioning of cells using ultrasonic forces).

Dr. Jens Sobek, Functional Genomic Center Zurich, Universität Zürich, Zürich, CH (antibody microarrays to analyse the activation state of cells).

13.1.3 Expenditure of Third Party Funds ("Total Costs" in SAP)

Main Applicant

SNF (3100A0-104144/1):

The multidomain protein CNK: a regulator of the kinase pathway and integrator of signaling pathways (6/2004 – 5/2006; CHF 125.392.-).

Krebsliga Zürich:

The role of Eph receptor tyrosine kinase in angiogenesis (5/2002 – 12/2004; CHF 105.000.-).

Schweizerische Krebsliga (1003-02-2000):

Identification of proteins interacting with the oncoprotein ErbB2 – role in signal transduction and tumorigenesis (6/2000 – 5/2003; CHF 145.700.-).

Co-Applicant

SNF (31-61965.00):

Signaling of the Raf kinase in complex biological systems (10/2001 – 9/2004; CHF 320.000.-).

SNF:

The role of the Raf kinase in normal and tumor cells during development and tumor formation (4/1997 – 3/2000; CHF 270.000.-).

13.1.4 Publications, 2000 - 2004

- Vollständige Publikationslisten z.B. in Anhang. Publikationsliste des/der Lehrstuhlinhabers/in und der Mitarbeitenden getrennt nach Erscheinungsjahr und folgenden Publikationsarten:

Articles in Academic/Scientific Journals

- (Bitte Publikationsliste nach folgenden Arten gruppieren – allenfalls den Usanzen des Fachs anzupassen.)
  - *Originalarbeiten*
    - *In peer reviewed journals / in referierten Zeitschriften*

18. Heinicke T.,* **Radziwill G.***, Nawrath M., Rommel C., Zimmermann S., Pavlovic J., and Moelling K. Retroviral gene transfer of dominant negative raf-1 mutants suppresses Ha-ras induced transformation and delays tumor formation. Cancer Gene Ther. 7, 697-706 (2000).

19. **Radziwill G.**, Erdmann R., Margelisch U., Moelling K. Bcr is a PDZ domain binding kinase that phosphorylates AF-6 and downregulates Ras/Raf signaling. Mol Cell Biol. 23, 4663-46672 (2003).

20. Boisguerin P., Leben R., Ay B., **Radziwill G.**, Moelling K., Liying D., Volkmer-Engert R. An Improved Method for the Synthesis of Cellulose Membrane-Bound Peptides with Free C-Termini Useful for PDZ Domain Binding Studies. Chemistry & Biology, 11, 449-459 (2004).

21. Ziogas A., Moelling K., and **Radziwill G.** The scaffold protein CNK connects Raf-1 and Src signaling J. Biol. Chem. 280, 24205-24211 (2005).

22. Haake A., Neild A., **Radziwill G.**, and Dual J. Positioning, displacement, and localization of cells using ultrasonic. Biotechnology and Bioengineering 92, 8-14 (2005).

23. Rafael F.D., and **Radziwill G.** The scaffold protein CNK1 interacts with the angiotensin II type 2 receptor. Biochem. Biophys. Res. Commun., in press.

- *Übersichtsartikel (reviews)*

Radziwill G. Records for MAP Kinases, ERK, JNK, p38. In: xPharm, Elsevier, www. mdl.com/products/knowledge/xpharm/index.jsp (2004)

(interactive database covering molecular targets, agents, related disorders, and principles that govern their interaction)

13.1.5 National and International Awards and Honors 2000 – 2004

13.1.6 Offers of a Professorship at Other Institutions 2000 – 2004

13.2 Teaching

13.2.1 List of the Diploma Theses That You Supervised

- Nach Abschlussjahr geordnet; mit Angabe von Titel, Jahr und Autorin / Autor.

Dag Schauwienold: Charakterisierung von Bindungspartnern des Raf-1 assoziierten Proteins CNK (2000).

Sandra Wechsler: Interaktion des DZ-Proteins AF-6 mit dem Transkriptionsfaktor MyT1 (2000).

Nadine Hardel: Das PDZ-Protein ERBIN bildet einen Komplex mit der Proteinkinase Bcr und der Protoonkogenprodukt beta-Catenin (2001).

Rafael Fritz: Interaktion zwischen dem Multidomänenprotein CNK und dem Angiotensinrezeptor Typ 2 (2005).

13.3 Next Generation of Academics/Scientists

13.3.1 List of Completed Dissertations and Habilitations

- chronologisch geordnet; mit Angabe von Titel und Autorin / Autor

Algirdas Ziogas: Characterisation of the multistep activation of the kinase Raf-1 involving phosphorylation and protein protein interaction (2001).

Rüdiger A. Erdmann: The role of the Bcr kinase and the PDZ domain protein AF-6 in signal transduction (2004).

13.4 Further Aspects

- Visiting Professors: Gastprofessuren von Angehörigen der Forschungsgruppe 1998-2002 an anderen Institutionen (minimale Dauer 2 Wochen)
- Editorships: Chief Editor (journals/series); Member of Editorial Boards / Associate Editor (journals/series)
- Offices / Functions Held at the University (self-administration) and outside
- Relationships to Government (Politics), the Economy, and Society

13.5 Curriculum Vitae

- CV mit Stationen des akademischen / beruflichen Werdegangs (ohne Auflistung der Publikationen).

Personal information

Name	Gerald Radziwill
Address	Institute of Medical Virology University of Zurich Gloriastr. 30 CH-8006 Zurich, Switzerland Tel: +41 1 634 2787 Fax: +41 1 634 4906 e-mail: radziwil@immv.unizh.ch
Born	14 August 1960, Freiburg i. Br., Germany
Nationality	German
Marital status	married, two children (born 1988 and 1990)

Education

1980	Abitur, Karlsruhe
1980-85	Study of Biology at the University of Heidelberg
1985	Diploma degree in biology
1985-89	Graduate student at the ZMBH, University of Heidelberg; Prof. Dr. Heinz Schaller
1989	PhD thesis

Research positions

1989	Postdoc at the ZMBH, University of Heidelberg; Prof. Dr. Heinz Schaller
1989-94	Postdoc at the Max-Planck Institute of Molecular Genetics, Berlin; Prof. Dr. Karin Moelling
1994-99	Group leader (Assistant) at the Institute of Medical Virology, University of Zurich (Director: Prof. Dr. Karin Moelling)

Since 1999 Senior group leader (Oberassistent) at the Institute of Medical Virology
University of Zurich (Director: Prof. Dr. Karin Moelling)

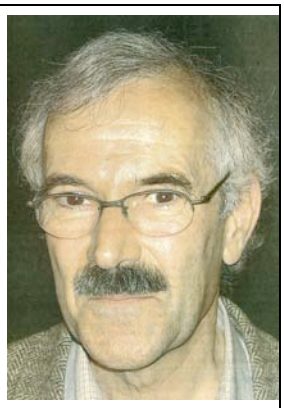
Memberships
Gesellschaft für Biochemie und Molekularbiologie e.V.
Signal Transduction Society (STS)
American Society for Biochemistry and Molecular Biology (ASBMB)

14 OA PD Dr. W. Bossart

14.1-14.4 Diagnostics, Research and Publication list of Dr. Bossart
see chapter 8

14.5 Curriculum vitae

Curriculum vitae



Person: **Bossart Walter**
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Studies:

1969-1973	Basic studies in biology, University of Basle, Switzerland.
1973	Masters degree in biology.
1973-1976	PhD thesis in virology, Institute of Microbiology (Prof. H. Loeffler, Prof. K. Bienz), University of Basle.
1976	PhD degree in biology.
1978-1984	Institute of Microbiology, University of Basle: Research in virology; (Tutor: Prof. K. Bienz, Referee: Prof. H. Eggers, Köln, Germany); diagnostics in general microbiology.
1984	"Privatdozent“ for medical microbiology, University of Basle.

Additional education:

1973	SKMB course in "Protein Biosynthesis" (Prof. T. Stäheli, Prof. M. Schreier), Roche Institute of Immunology, Basle, Switzerland).
1977/1978	Post-doctoral fellowship at SUNY Albany N.Y. (Dr. D.L. Nuss, Dr. E. Paoletti, Prof. C. Baglioni), sponsored by a grant of the Swiss National Science Foundation.
1980	NATO Summer School on Protein Biosynthesis, Marathea, Italy.
1986	Training in project management (Business School St. Gallen, Switzerland).
1986	Training in academic teaching (Prof. Goldschmid, University of Lausanne, Switzerland).
1989	Basic management training (E. Krauthammer SA).
1991	Course in "Principles of Contemporary Immunology", (The Center for Professional Advancement; Dr. D.H. Sussdorf, Dr. L.R. Draper, Dr. Ch. Wood), Amsterdam, The Netherlands.
1992	Swiss Federal FAMH qualification in medical microbiology; authorisation to head of a diagnostic microbiology laboratory.

Positions and Functions:

1973-1977 and 1978-1984	Institute of Medical Microbiology (Prof. H. Loeffler, Prof. K. Bienz, Prof. P. Erb), University of Basle, Switzerland; involved in research, teaching and diagnostics.
Since 1984	“Privatdozent” at the University of Basle, limited teaching obligations.
1984-1993	Pharmaceutical industry (Solco Basle Ltd.); responsible for development and production of bacterial vaccines / immunomodulators; 2 years head "ad interim" of Solco's Research Department.
Since 1994	Institute of Medical Virology (Prof. K. Moelling), University of Zurich, Switzerland: Head of virus diagnostic laboratories; involved in teaching and research.
Since 2000	Council member for Switzerland in the Council of the European Society for Clinical Virology (ESCV).
Since 2005	Expert for virology, serology and molecular diagnostics on contract of the Swiss Accreditation Service.

Membership in Societies:

Since:	Society:
1975	Swiss Society of Microbiology (SGM/SSM)
1994	Society of Virology (GfV) (Austrian-German-Swiss society)
1997	European Society for Clinical Virology (ESCV)
1998	American Society of Microbiology (ASM)

Part C: Appendix

Lecture-Index, Lectures University

Dozentinnen und Dozenten mit Adressen und Vorlesungen

Missfelder Jan-Friedrich,
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Kurse, Kolloquien und Repetitorien

Mikrobiologischer Kurs: Teil Medizinische Mikrobiologie
514 T Mo 13–15 Mo 15–17 Di 16–18 Mi 13–15 Mi 15–17 Do 16–18
Burkhard Springer

Virologisches Kolloquium Ausgewählte Kapitel Virologie, Onkologie, Gentherapie
515 n. Ankünd.
Karin Mölling

Aktuelle Probleme der Immunologie und Virologie
516 Di 12–13
Hans Hengartner, Karin Mölling, Rolf M. Zinkernagel, Thomas Martin Kündig, Jovan Pavlovic

Diskussion aktueller Forschungsprojekte
517 Do 16.30 –17.15 alle 14 Tage
Brigitte Berger–Bächi, Peter Sander

Methoden der Mikroskopie
518 Mi 8–10 alle 14 Tage
Thomas Bächi

Konfokale Mikroskopie (Theorie und Praktikum)
519 9.–13.2.
Thomas Bächi

Arbeiten im Laboratorium für Mikrobiologie
520 täglich
Martin Altwegg, Peter Sander, Burkhard Springer

Arbeiten im Laboratorium für Virologie
521 täglich
Karin Mölling, Jovan Pavlovic

Kolloquium über mikroskopische Strukturanalysen
522 Di 8– 9
Thomas Bächi

Mikroskopiekurs für Fortgeschrittene
523 I 1 Woche in den Frühlingsferien
Thomas Bächi, Robert Stidwill

Individuelle Praktika nach Absprache: Pathogenität, Resistenz, Diagnostik
524 4 Wochen
Martin Altwegg, Reinhard Zbinden, Peter Sander, Burkhard Springer

Pharmakologie

Pharmakologie und Toxikologie, I. Teil
525 T Mo 8–10 Di 8–10
Alexander A. Borbély, Jean–Marc Fritschy, Uwe Rudolph, Kaspar Vogt

Pharmakotherapie (Leitung: A. Borbély und P. Meier–Abt)
526 T Do 11–12.30
Alexander A. Borbély, Ferenc Follath, Michael Fried, Beat Michel, Heinz–Gregor Wieser, Brigitte Woggon, Peter Greminger, Rainer Weber

Kurse, Kolloquien und Repetitorien

Praktikum in Pharmakologie
527 I Block
Alexander A. Borbély, Hanns Möhler, Jean–Marc Fritschy, Uwe Rudolph, Kaspar Vogt, Irène Tobler–Kost, Peter Achermann, Hans–Peter Landolt

Pharmakologisches Seminar
528 I Mi 17–18
Alexander A. Borbély, Hanns Möhler, Jean–Marc Fritschy, Uwe Rudolph, Kaspar Vogt, Irène Tobler–Kost, Peter Achermann, Hans–Peter Landolt

Doktorandenkolloquium
529 I Do 9–10
Alexander A. Borbély, Hanns Möhler, Jean–Marc Fritschy, Uwe Rudolph, Kaspar Vogt

Arbeiten im Laboratorium
530 I täglich
Alexander A. Borbély, Hanns Möhler, Jean–Marc Fritschy, Uwe Rudolph, Kaspar Vogt, Peter Achermann, Hans–Peter Landolt

Ärztliche Fortbildung

Aktuelle Probleme der Krankheitsforschung
801 Mo 17–18
Adriano Aguzzi, Hans Hengartner, Rolf M. Zinkernagel, Bernhard Odermatt

Kolloquium: Klinik und Mikrobiologie von Infektionskrankheiten
802 Do 17.00–17.45
Erik Christian Böttger, Peter Deplazes, Karin Mölling, Peter Köhler, David Nadal, Walter Siegenthaler, Christian Ruef, Rainer Weber, Reinhard Zbinden

Medizinische Mikrobiologie: Themen aus Diagnostik und Forschung
803 1x im Monat n. Ankünd. Di 16.30 18.00
Martin Altwegg, Reinhard Zbinden, Jochen Gottschalk, Peter Sander, Burkhard Springer

Infektiologische Fallbesprechungen
804 Di 12–13
Erik Christian Böttger, David Nadal, Martin Krause, Milos Opravil, Christian Ruef, Rainer Weber, Huldrych Günthard, Bruno Ledergerber, Roberto Speck, Urs Karrer, Nicolas Müller

Aktuelle Probleme der Infektiologie
805 Mo 12–13
David Nadal, Milos Opravil, Christian Ruef, Jörg Schüpbach, Rainer Weber, Jürg Böni, Marek Fischer, Huldrych Günthard, Bruno Ledergerber, Roberto Speck, Alexandra Trkola, Markus Flepp, Josef Jost, Urs Karrer, Nicolas Müller

Kolloquium für Tropen- und Reisemedizin
806 Mo 11.30 –12.15
Wilhelm Vetter, Robert Steffen, Rainer Weber

Medizinisch-pathologisch-anatomisches Kolloquium
807 Do 14–15
Adriano Aguzzi, Ferenc Follath, Michae Fried, Andreas Schaffner, Oswald Oelz, Rudolf Speich, Peter Bauerfeind, Marcc Maggiorini, Franco Salomon mit Oberärzten und Assistenten

Interdisziplinäre Fallbesprechungen von Familien mit vererbter Krebsprädispositio
808 erster Mo im Monat
Rolf Stahel, Peter Bauerfeind, Judit Pok Lundquist

Interdisziplinäre Tumorfall-Besprechung im Stadtspital Triemli (Pathologie, Stock y, Zi 131)
809 Mi 17.00–18
Hanspeter Honegger, Robert Maurer, Urs Metzger

Interdisziplinäre onkologische Fallbesprechung
810 Di 14–15
Urs Martin Lütolf, Rolf Stahel, Pia Huguenin

Kardiologische Fallbesprechungen
811 Di 12.30–13.30
Thomas F. Lüscher, Rolf Jenni, Philipp Kaufmann, Juraj Turina, Firat Duru, Willibald Maier, Erwin Notker Oechslin, Jürg Schwitter, Roberto Corti, Bernd van der Loo

Cardiology Rounds: Kardiologische Fortbildung für Ärzte
812 Do 18–19
Thomas F. Lüscher, Rolf Jenni, Philipp Kaufmann, Firat Duru

Kardiologische Forschungskonferenz
813 1 Stunde gem. Ankünd.
Thomas F. Lüscher, Felix C. Tanner, Francesco Cosentino, Christian Matter

Rehabilitationsmedizin und Rehabilitationspsychologie
973 Blockkurs 1 Woche
Claus Buddeberg, Peter Risi, Stephan Spiess

Drogenkolloquium
974 1. Mi/Monat 9–10
Wulf Rössler, Dominique Eich–Höchli, Rudolf Stohler

Substitutionskolloquium
975 n. Ankünd.
Wulf Rössler, Dominique Eich–Höchli, Rudolf Stohler

Einführung in die Substitutionsbehandlung (Grundkurs)
976 n. Ankünd.
Wulf Rössler, Dominique Eich–Höchli, Rudolf Stohler

Sozialpsychiatrisches Kolloquium (Militärstr. 8)
977 Do 13–14.30
Wulf Rössler, Roland Buchser

Kolloquium sozialpsychiatrischer Forschungsprojekte (Militärstr. 8)
978 n. Ankünd.
Wulf Rössler, Vladeta Ajdacic, Christoph Lauber

Forschungskonferenz
979 n. Ankünd.
Wulf Rössler, Dominique Eich–Höchli, Rudolf Stohler, Christoph Lauber

Gehirn und Verhalten, für Mediziner, Psychologen und Biologen
980 Mi 14–15
Martha Lehmann–Koukkou

Psychobiologische Modelle des menschlichen Gehirns und die menschlichen Emotionen
981 Mi 15–16
Martha Lehmann–Koukkou

Einführung in die Testdiagnostik für Assistenzärzte I (Anfänger)
982 Do 8–12 alle 14 Tage
Christoph Käppler

Trainingskurs in Gesprächsführung (während den Semesterferien)
983 gem. Ankünd.
Karl Dülli–Loher, Hadmut Prün, Christoph Walder

Probleme der Fahreignung
751 I Di 13–14
Walter Bär

Rechtsmedizinisches Kolloquium
752 I Do 13.30–14.15 alle 14 Tage
Walter Bär und Oberärzte

Diagnostik an alten Skelettfunden – Klinischpaläopathologisches Kolloquium
759 Di 16–18 alle 14 Tage
Jürg Hodler, Arthur von Hochstetter, Thomas Böni, Frank Jakobus Rühli

Medizinhistorische Vortragsreihe (für Hörerinnen und Hörer aller Fakultäten)
984 Do 18.15–19.45 1 mal monatlich
Beat Rüttimann, Christoph Mörgeli

Medizinische Museologie: Erarbeiten einer Ausstellung
985 Do 15–17
Christoph Mörgeli

Postgraduate Kurs für experimentelle Medizin und Biologie (Leitung: J. Zapf)
986 nach Absprache
Kurt Bürki, Hans Hengartner, Karin Mölling, Andreas Plückthun, Jürgen Roth, Jürgen Zapf, Rolf M. Zinkernagel, Kurt Blaser, Thomas Bächi, Jürg Biber, Bruno Stieger, Peter Streit, Cezmi Ali Akdis, Thierry Hennet, Rolf Jaussi, Jovan Pavlovic, Peter Lindner, Hans–Rudolf Roth

Praktischer Kurs in Immunologie für Teilnehmende des Postgraduate-Kurses, Studierende der Molekularbiologie und Biochemie
987 T E 30.8.–10.9. 13.9.–24.9.
Rolf M. Zinkernagel, Kurt Blaser, Karl Frei Leitung: H. Hengartner

BIO 282 Pflanzenbiologischer Methodenkurs
2851 T 4.–7. Woche
Enrico Martinoia, Robert Dudler, Felix Keller, Markus Curtis, Christoph Ringli

BIO 283 Molekulare Pflanzenphysiologie: Abiotischer Stress
2852 T 8.–11. Woche
Enrico Martinoia, Markus Geisler, Markus Klein

BIO 284 Interaktionen zwischen Bakterien in Biofilmen
2853 E 8.–11. Woche
Leo Eberl, Kathrin Riedel

BIO 285 Pflanzen-Entwicklungsbiologie
2854 T 11.–14. Woche
Ueli Grossniklaus, Markus Curtis, Claudia Köhler, Enrico Perotti

BIO 293 Evolution und Ökologie der Mikroorganismen
2855 T O 4.–7. Woche
Leo Eberl, Kurt Hanselmann

BIO 298.2 Mykologie (doppelt geführt)
2856 T E 5.–7. Woche
Markus Aebi, Adrian Leuchtmann, Rosmarie Honegger

BIO 298.3 Mykologie (doppelt geführt)
2857 E 12.–14. Woche
Markus Aebi, Adrian Leuchtmann, Rosmarie Honegger

BIO 300.2 Mikrobielle Genetik (doppelt geführt)
2858 T E 5.–7. Woche
Hauke Hennecke, Wolf-Dietrich Hardt, Hubert Hilbi, Linda Thöny-Meyer

BIO 300.3 Mikrobielle Genetik (doppelt geführt)
2859 E 12.–14. Woche
Hauke Hennecke, Wolf-Dietrich Hardt, Hubert Hilbi, Linda Thöny-Meyer

BIO 310.2 Mikrobielle Gentechnologie (Praktikum ETHZ)
2860 E Woche 1–3 der Semesterferien
Hans-Martin Fischer, Pauli Kallio, Linda Thöny-Meyer

BIO 312 Geobiologische Exkursionen
2861 24.9.–3.10.
Hugo F. R. Bucher, Heinz Furrer, Kurt Hanselmann, Helmut Weissert

BIO 321 Struktur und Dynamik der Zelle
2862 T I 1.–4. Woche
Jürgen Roth, Urs Greber, Thierry Hennet, Jack Rohrer, Martin Ziak, Christian Zuber

BIO 322 Zellbiologie viraler Infektionen
2863 T I 4.–7. Woche
Karin Mölling, Urs Greber, Cornel Fraefel, Jovan Pavlovic, Silvio Hemmi

BIO 323 Moderne Genetik und Genomik
2864 T I 8.–14. Woche
Wolfgang Berger, Ernst Hafen, Monica Steinmann-Zwicky, Peter Gallant, Alex Hajnal, Daniel Bopp

BIO 324 Verhaltensbiologie
2865 I 1.–7. Woche
Barbara König, Marta Manser, Dennis C. Turner

BIO 361 Reproduktionsbiologie
2866 I 1.–7. Woche
Kurt Bürki, Ueli Grossniklaus, Paul Ward, Eric Kubli, Georg Ribi, Wolf Blanckenhorn, Thomas Rülicke, Thomas Georg Honegger, Claudia Köhler

BIO 362 Evolutionäre Genetik
2867 T I Montag ganztägig
Paul Ward, Georg Ribi, Wolf Blanckenhorn

BIO 353 Vogelzug und Vogelflug
2868 T 5 ganze Tage (Woche 41)
Lukas Jenni

BIO 401 Funktionen des Körpers: Vegetative Systeme
2869 T I 1.–7. Woche
Eric G. Berger, Heini Murer, Roland H. Wenger, François Verrey, Bruno Stieger, Thierry Hennet

Topics Lecture Virology and Course

Programm der Vorlesungen und Kurse, SOMMERSEMESTER 2005:
Mikrobiologie, Virologie, Immunologie, Parasitologie

Gruppeneinteilung Kurs

Gruppe A:	Montag	13.00-14.45 h
	Mittwoch	13.00-14.45 h
Gruppe B:	Montag	15.15-17.00 h
	Mittwoch	15.15-17.00 h
Gruppe C:	Dienstag	16.15-18.00 h
	Donnerstag	16.15-18.00 h

Kursraum Mikrobiologie: Gloriastr. 30, Raum GLM E18 a/b/c

Tag:	Datum:	Zeit:	Fach:	Thema:	Dozent:	Ort:
Mittwoch	25.05.2005	11.15-12.00	Vorlesung Virologie 1	Struktur und Replikation	Prof. K. Mölling	Gr. Hörsaal D-Nor
Montag	30.05.2005	11.15-12.00	Vorlesung Virologie 2	Virus-Wirtszell-Wechselwirkung	Prof. K. Mölling	Gr. Hörsaal D-Nor
Mittwoch	01.06.2005	11.15-12.00	Vorlesung Virologie 3	Influenzaviren	Prof. K. Mölling	Gr. Hörsaal D-Nor
Montag	06.06.2005	11.15-12.00	Vorlesung Virologie 4	Virusdiagnostik	Prof. K. Mölling PD Dr. W. Bossart	Gr. Hörsaal D-Nor
			Kurs Virologie I			
Montag	06.06.2005	13.00-14.45	Gruppe A		Prof. K. Mölling	Kursraum
Montag	06.06.2005	15.15-17.00	Gruppe B		PD Dr. W. Bossart	Mikrobiologie
Dienstag	07.06.2005	16.15-18.00	Gruppe C		PD Dr. J. Pavlovic	
Mittwoch	08.06.2005	11.15-12.00	Vorlesung Virologie 5	Herpesviren	Prof. K. Mölling	Gr. Hörsaal D-Nor
			Kurs Virologie II			
Mittwoch	08.06.2005	13.00-14.45	Gruppe A		Prof. K. Mölling	Kursraum
Mittwoch	08.06.2005	15.15-17.00	Gruppe B		PD Dr. W. Bossart	Mikrobiologie
Donnerstag	09.06.2005	16.15-18.00	Gruppe C		PD Dr. J. Pavlovic	
Montag	13.06.2005	11.15-12.00	Vorlesung Virologie 6	Schwangerschaft und Kinderkrankheiten	Prof. K. Mölling	Gr. Hörsaal D-Nor
Mittwoch	15.06.2005	11.15-12.00	Vorlesung Virologie 7	Hepatitis Viren	Prof. K. Mölling	Gr. Hörsaal D-Nor
Montag	20.06.2005	11.15-12.00	Vorlesung Virologie 8	Humane Retroviren	Prof. K. Mölling	Gr. Hörsaal D-Nor
Mittwoch	22.06.2005	11.15-12.00	Vorlesung Virologie 9	Entero- / Adenoviren	Prof. K. Mölling	Gr. Hörsaal D-Nor
Montag	27.06.2005	11.15-12.00	Vorlesung Virologie 10	Antivirale Substanzen Impfungen	Prof. K. Mölling	Gr. Hörsaal D-Nor
Mittwoch	29.06.2005	11.15-12.00	Vorlesung Virologie 11	Tumorviren / Gentherapie	Prof. K. Mölling	Gr. Hörsaal D-Nor
Samstag	02.07.2005		**Abschluss Sommersemester**			

INSTITUT FÜR EXPERIMENTELLE IMMUNOLOGIE UND
INSTITUT FÜR MEDIZINISCHE VIROLOGIE

Vorlesungsreihe Nr. 516 / Wintersemester 2004/2005

AKTUELLE PROBLEME DER IMMUNOLOGIE UND VIROLOGIE

Gastgeber:	H. Hengartner, K. Moelling, A. Oxenius, R. Zinkernagel
Zeit:	Dienstag, 12:15 Uhr
Ort:	**kleiner Hörsaal Pathologie / Universitätsspital (C PATH 22)**

19.10.2004	Dr. Sameh Basta University of Constance, Biology Dept. /Division of Immunology **"Cross-Presentation of viral antigens & induction of cellular immune responses"** (Institut für Experimentelle Immunologie)
26.10.2004	Dr. Markus Manz Institute for Research in Biomedicine, Bellinzona **"Immunoreconstitution of mice and men"** (Prof. Annette Oxenius, Institut für Mikrobiologie, ETH Zürich)
02.11.2004	Prof. Antonio Lanzavecchia Institute for Research in Biomedicine, Bellinzona **"Exploring and exploiting human B cell memory"** (Institut für Experimentelle Immunologie)
9.11.2004	Dr. Steffen Jung Weizmann Institute, Dept. of Immunology Rehovot, Israel **"Origins and Functions of the Mononuclear Phagocyte System"** (Institut für Experimentelle Immunologie)
16.11.2004	Dr. Markus H. Gräler Medical School Hannover, Institute for Immunology "**FTY720, Sphingosine 1-Phospate, and the S1P1-Receptor: Important Pieces for Solving the Puzzle of Lymphocyte Recirculation"** (Institut für Experimentelle Immunologie)

Each host invites 1-2 speakers per semester (total 13 speakers).

Retroviren, Onkogene, Signaltransduktion in normalen und Tumorzellen, Gentherapie mit Virus-Vektoren

PG-Kurs für experimentelle Medizin und Biologie
WS 2004/2005, Nr. 986

Prof. Dr. K. Mölling und Mitarbeiter
Institut für Medizinische Virologie

Dienstag / Mittwoch, 2./3. November 2004

Hörsaal: Uni Zürich Zentrum (UZZ), Gebäude HIM, Pavillon A

2.11.2004	**09.15 Uhr**	**(1, 2)**	**Retroviren**
		(3)	**Onkogene, Tumorsuppressoren**
		(4)	**Signaltransduktion via Kinasen**
		(5, 6)	**HIV**
	17.00 Uhr ca.	**Ende**	
3.11.2004	**09.15 Uhr**	**(7)**	**Virus-Rezeptoren**
		(8)	**Molekulare Virus-Diagnostik**
		(9)	**Virus-Vekoren für Gentherapie**
		(10)	**SiRNA Technologie**
	17.00 Uhr ca.	**Ende**	

Literatur:

Molekular Onkologie, Christoph Wegener, Thieme 1999.

(1) S.A. Matheny et. al: Ras regulates assembly of mitogenic signalling complexes through the effector protein IMP. *Nature* **427**: 256-260 (2004).
(2) A. Alavi et al: Role of Raf in vascular protection from distinct apoptotic stimuli. *Science* **301**: 94-96 (2003).
(3) T. Reya et al: A role for Wnt signalling in self-renewal of haematopoietic stem cells. *Nature* **423**: 409-414 (2003).
(4) D.N. Chadee et al: MLK3 is required for mitogen activation of B-Raf, ERK and cell proliferation. *Nature Cell Biology* **6**: 770-776 (2004).
(5) S. Swingler et al: HIV-1 Nef intersects the macrophage CD40L signalling pathway to promote resting-cell infection. *Nature* **424**: 213-219 (2003).
(6) H. Zhang et al: The cytidine deaminase CEM15 induces hypermutation in newly synthesized HIV-1 DNA. *Nature* **424**: 94-98 (2003).
(7) M. Shimojima et al: Use of CD134 as a primary receptor by the feline immunodeficiency virus. *Science* **303**: 1192-1195 (2004).
(8) Y-L. Chiu et al: Inhibition of human immunodeficiency virus type 1 replication by RNA interference directed against human transcription elongation factor P-TEFb (CDK9/CyclinT1). *Journal of Virology* **78**: 2517-2529 (2004).
(9) C. Lois et al: Germline transmission and tissue-specific expression of transgenes delivered by leniviral vectors. *Science* **295**: 868-872 (2002).
(10) J.-M. Jacque et al: Modulation of HIV-1 replication by RNA interference. *Nature* **418**: 435-438 (2002).

Up to 70 students totally have participated in the practical laboratory course at the IMV.

Lecture „Studiengang Mikrobiologie“, Uni / ETH

Vorlesungsverzeichnis
Lerneinheiten
erweiterte Sicht
Übersicht
Druckversion
Dozierende
Detailsuche
Gesamtverzeichnis

Suchergebnis der Lerneinheiten im Sommersemester 2005

erweiterte Sicht 4 LE

Nummer	Name der Lerneinheit (Kreditpunkte)	Angaben zum Studienplan	Dozent/in	

Biologie

Einleitung Legende Legende "Angaben zum Studienplan"

III. Fachstudium: Obl. Lehrveranstaltungen nach Fachrichtung

6. und 8. Semester, Testatpflicht für U, G, P, S

Prüfungsfächer im Schlussdiplom: Bezeichnung, zugehörende Lehrveranstaltungen und Prüfungsumfang siehe Wegleitung Obligatorische Prüfungsfächer: Die zugehörenden Lehrveranstaltungen sind bei den zutreffenden Fachrichtungen aufgeführt Wahlfächer: Wahlfachempfehlungen siehe Wegleitung, bzw. Beratervorschläge; Lehrveranstaltungen zu den Prüfungsfächern für alle Fachrichtungen im Sommersemester siehe im Abschnitt IV

Fachrichtung 3: Mikrobiologie

6. Semester

551-1108-00L	Praktikum Experimentelle Mikrobiologie II (20 KP)	O/Dr	Loessner, M.	Detail »
551-1134-00L	Molekulare Virologie (2 KP)	S*/Dr	Mölling, K.	Detail »

Fachrichtung 6: Biochemie und Molekularbiologie

6. Semester

551-1108-00L	Praktikum Experimentelle Mikrobiologie II (20 KP)	O*/Dr	Loessner, M.	Detail »

IV. Lehrveranstaltungen zu den Prüfungsfächern im Schlussdiplom für alle Studienrichtungen

Prüfungsfächer und zugehörende Lehrveranstaltungen siehe Wegleitung

6. Semester oder ausnahmsweise 8. Semester

Med. Mikrobiologie/Virologie/Parasitologie

551-1134-00L	Molekulare Virologie (2 KP)	WS/Dr	Mölling, K.	Detail »

erweiterte Sicht 4 LE

Details einer Lerneinheit

Allgemeines

Titel	Molekulare Virologie
Nummer	551-1134-00L
Semester	SS 2005
Dozierende	**Mölling, K.** | Pavlovic, J. | Radziwill, G. | Weiss, A.
Periodizität	jährlich wiederkehrende Veranstaltung
Sprache	Deutsch
Leistungskontrolle	Hier finden Sie weitere Angaben.

Zugehörige Lehrveranstaltungen

Nummer	Typ	Lehrveranstaltung	Std.	Dozent/in	Tag	Zeit	Lokal
551-1134-00	V	Medizinische Virologie Kurs an der UNI Zürich, Montag 11-12h, Gloriastr. 32, GLP 1 206.	2	**Mölling, K.** Pavlovic, J. Radziwill, G. Weiss, A.	Mo Mi	11-12 11-12	 ML F 38 »

Katalogdaten Originalsprache

Kurzbeschreibung

Inhalt

Viren sind Minimalisten, die mit nur wenigen Genen ihr überleben garantieren. Sie haben dazu während der Evolution - die bis heute andauert - raffinierte Strategien und molekulare Tricks entwickelt und sind die intimsten Kenner ihrer Wirtszelle. Viele molekulare Vorgänge, wie z.B. das Spleißen, wurden zuerst durch Viren entdeckt. Da sie sich gentechnisch zerlegen und neu rekonstituieren lassen, sind sie zum bedeutenden Werkzeug der Molekularbiologen geworden. Sie bieten die Möglichkeit zur Analyse nicht nur der eigenen, sondern auch der Gene der Wirtszelle oder des Wirtsorganismus. Sie können über Krebsgene zur Tumorentstehung beitragen - aber auch die Umkehrung ist möglich - Viren mit Thearpiegenen werden als Vehikel zur Gentherapie eingesetzt.
Es werden in der Vorlesung virale Strategien und Prinzipien behandelt, einschließlich der molekulare Vorgänge der Replikation, Eigenschaften von Onkogenen und Tumorsupressorgenen, Pathogenitätsmechanismen, Krebsentstehung, Ansatzmöglichkeiten für Therapien, Gentherapie und Biotechnologie. Einige Viren wie z.B. HIV Influenzaviren Hepatitisviren, Herpesviren und andere neurotrope Viren, werden besonders abgehandelt.

Lernziel

Viren sind Minimalisten, die mit nur wenigen Genen ihr überleben garantieren. Sie haben dazu während der Evolution - die bis heute andauert - raffinierte Strategien und molekulare Tricks entwickelt und sind die intimsten Kenner ihrer Wirtszelle. Viele molekulare Vorgänge, wie z.B. das Spleißen, wurden zuerst durch Viren entdeckt. Da sie sich gentechnisch zerlegen und neu rekonstituieren lassen, sind sie zum bedeutenden Werkzeug der Molekularbiologen geworden. Sie bieten die Möglichkeit zur Analyse nicht nur der eigenen, sondern auch der Gene der Wirtszelle oder des Wirtsorganismus. Sie können über Krebsgene zur Tumorentstehung beitragen - aber auch die Umkehrung ist möglich - Viren mit Thearpiegenen werden als Vehikel zur Gentherapie eingesetzt.

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Skript Unterlagen werden verteilt

Literatur

- Flint S:J, Enquist L.W., Krug R.M., Racaniello V.R. und Skalka A.M.: Principles of Virology, ASM Press, 2000 (anspruchsvoll)
- Wagener C.: Molekulare Onkologie (2. Auflage) Thieme Verlag Stuttgart,1999
- Modrow S. und Falke, D.: Molekulare Virologie. Spektrum Akademischer Verlag, Heidelberg , 1997
- Coffin J.M., Hughes, S.H. und Varmus H.E.: Retroviruses. Cold Spring Habor Laboratory Press, 1997

Katalogdaten Englisch

Titel

Kurzbeschreibung

angeboten in

Studiengang	Bereich	Angaben zum Studienplan	
Biologie	6. Semester	S*/Dr	Legende
Biologie	Med. Mikrobiologie/Virologie/ Parasitologie	WS/Dr	Legende

Information zur Leistungskontrolle für Bachelor- und Masterstudiengänge - Leistungskontrolle als Semesterkurs

Kreditpunkte* 2

Prüfende **Pavlovic, J.**, Mölling, K., Radziwill, G., Weiss, A.

Form unbenotete Semesterleistung

Sprache Deutsch

Testat erforderlich Ja

* Falls die Lerneinheit innerhalb eines Prüfungsblockes geprüft wird, werden die Kreditpunkte für den gesamten bestandenen Block erteilt.

Diese Angaben können noch zu Semesterbeginn aktualisiert werden; verbindlich sind die Angaben auf dem Prüfungsplan.

Topics Lecture „Studiengang Mikrobiologie“ Uni / ETH

Sommersemester 2004 (total 28 Std.)

(1)	30.03.	Retroviren / HIV
(2)	04.04.	Retroviren / HIV
(3)	06.04.	Akut und chronisch transformierende Retroviren
(4)	11.04.	zelluläre und Virus-induzierte Signaltransduktion
(5)	13.04.	zelluläre und Virus-induzierte Signaltransduktion
(6)	18.04.	Interaktion zwischen viralen Proteinen und zellulären Signalmolekülen
(7)	20.04.	Influenza
(8)	25.04.	Influenza
(9)	27.04.	Transformation und Tumorgenese
(10)	02.05.	Tumorsuppressoren und Apoptose
(11)	04.05.	Inhibition der Tumorsuppression durch virale Protein
(12)	09.05.	Inhibition der Apoptose durch virale Proteine
(13)	11.05.	Viren und Interferonsystem
(14)	18.05.	Virusrezeptoren / Viruseintritt
(15)	23.05.	Virusausbreitung/Zell-Zell-Kontakte
(16)	25.05.	Struktur und Replikation
(17)	30.05.	Virus-Wirt Wechselwirkung
(18)	01.06.	Gentherapie mit viralen Vektoren
(19)	06.06.	Gentherapie mit viralen Vektoren
(20)	08.06.	HSV
(21)	13.06.	Hepatitis
(22)	15.06.	Tumorviren
(23)	20.06.	seltene Viren
(24-28)	22./27./29.06.	Referate (10 Biologie-Studenten)

Literatur:

(1) S.A. Matheny et al. Ras regulates assembly of mitogenic signaling complexes through the effector protein IMP. *Nature* **427**: 256-260 (2004).

(2) A. Alavi et al. Role of Raf in vascular protection from distinct apoptotic stimuli. *Science* **301**: 94-96 (2003).

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(4) D.N. Chadee et al. MLK3 is required for mitogen activation of B-Raf, ERK and cell proliferation. *Nature Cell Biology* **6**: 770-776 (2004).

(5) T.P. Newsome, N. Scaplehorn, M. Way . SRC mediates a switch from microtubule- to actin-based motility of vaccinia virus. *Science* **306**:124-129 (2004).

(6) K. Raj et al. Virus-mediated killing of cells that lack p53 activity. *Nature* **412**:914-917 (2001).

(7) X. Wang X. Epidermal growth factor receptor is a cellular receptor for human cytomegalovirus. *Nature* 424:456-461 (2003).

(8) C. Lois et al. Germline transmission and tissue-specific expression of transgenes delivered by leniviral vectors. *Science* **295**: 868-872 (2002).

(9) D. Kobasa et al. Enhanced virulence of influenza A viruses with the haemagglutinin of the 1918 pandemic virus. *Nature* **431**:703-707 (2004).

(10) J.-M. Jacque et al. Modulation of HIV-1 replication by RNA interference. *Nature* **418**: 435-438 (2002).

(11) H. Zhang et al. The cytidine deaminase CEM15 induces hypermutation in newly synthesized HIV-1 DNA. *Nature* **424**: 94-98 (2003).

(12) M.N. Poy et al. A pancreatic islet-specific microRNA regulates insulin secretion. *Nature* **432**: 226-230 (2004).

(13) S. Pfeffer et al. Identification of virus-encoded microRNAs. *Science* **304**: 734-736 (2004).

Index Lecture Course FU-Berlin

Ergebnis der Suche im Vorlesungsverzeichnis Wintersemester 2003 / 2004
Alle Fachbereiche und Zentraleinrichtungen

Veranstaltungen aller Fachbereiche und Zentraleinrichtungen

Biologie/Chemie/Pharmazie > Chemie, Biochemie > Biochemie (BC) > BC 2: Diplom Biochemie (Hauptstudium)

21 642a **- V -**	**Regulation der Genexpression durch Onkogene und Viren und Intervention durch Gentherapie** Vorlesungstermine: 12.12., 16.1., 13.2, jeweils 15.15-19.00 Vorlesung / Seminar: insgesamt 1 SWS (1,5 ECTS-Punkte). - Thielallee 63; Hs		Karin **Mölling**

1. Inhalt:
Signaltransduktion in normalen und Tumorzellen, Protein Kinase Kaskaden, Transkriptionsregulation, Proteindomänen, Modulation durch Phosphorylierung, Protein-Protein-Interaktion

Onkogene von Viren und zelluläre Onkogene, Tumorsuppressororgane, Multifaktorielle Krebsentstehung

Viren, molekulare Mechanismen der Replikation, Pathogenese, Schwerpunkt HIV,
Gentherapie (Ribozyme, Antisense, Triplex, Immunmodulatoren, Suizidgene, Viren als Vektoren)

2. Literatur:
Molekulare Onkologie, Christoph Wagener, Thieme Verlag (1999)
Molekulare Virologie, S. Modrow u. D. Falke, Spektrumverlag (2003)

3. Weitere Bemerkungen:
Möglichkeiten zur Labormitarbeit sind in Zürich (Institut für Medizinische Virologie) gegeben (s. 21 642c), (2 Monate nach Absprache)

Prof. Dr. K. Mölling: moelling@immv.unizh.ch

Biologie/Chemie/Pharmazie > Chemie, Biochemie > Biochemie (BC) > BC 2: Diplom Biochemie (Hauptstudium)

21 642b **- S -**	**Seminar zur Vorlesung 21642a** Vorlesung: ein Freitag pro Monat s. Ankündigung, jeweils 15.15-19.00 - Thielallee 63; Hs		Karin **Mölling**

Vorlesung / Seminar: insgesamt 1 SWS (1,5 ECTS-Punkte).

1. Inhalt:
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Biologie/Chemie/Pharmazie > Chemie, Biochemie > Biochemie (BC) > BC 2: Diplom Biochemie (Hauptstudium)

Laboratory Course in Zurich for Students from the FU-Berlin

21 642c **- P -**	**Mitarbeitspraktikum Signaltransduktion in normalen und Tumorzellen, Viren und Gentherapie für Naturwissenschaftler** 2 Monate ganztägig im Institut für Medizinische Virologie in Zürich, nach Absprache (Tel.: 0041-1-6342652), E-mail: moelling@immv.unizh.ch jederzeit nach Absprache, Vorb. 20.10., 9.00 Uhr, Hs, Thielallee 63		Karin **Mölling**

Mitarbeiten max. 8 SWS
ECTS s. Beschreibung

4 Wochen = 8 ECTS-Punkte
6 Wochen = 11 ECTS-Punkte
8 Wochen = 13 ECTS-Punkte
10 Wochen = 15 ECTS-Punkte
12 Wochen = 17 ECTS-Punkte

1. Inhalt (contents):
Onkogene, Tumorsuppressor, Kinase Kaskaden, Gengregulation, Viren, Gentherapie, Molekulare Mechanismen der Krebsentstehung

2. Literatur (literature):
-Ch. Wagner: Molekulare Onkologie, Thieme Verlag, Stuttgart (99)
- Modrow/Falke: Molekulare Virologie, Spektrum, Akad. Verlag (2003)

3 Weitere Bemerkungen (further comments)
auch für Bioinformatiker

4. Beginn (beginning): nach Absprache

Prof. Dr. K. Mölling: moelling@immv.unizh.ch

INSTITUT FÜR MEDIZINISCHE VIROLOGIE DER UNIVERSITÄT ZÜRICH

Direktion: Prof. Dr. Karin Mölling

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Gloriastrasse 30/32 Postfach CH-8028 Zürich

Vorlesung/Seminar
WE5, Berlin, Fachbereich Chemie
WS 2000/2001 - 21984 V/S

Prof. Dr. Karin Mölling

Molekulare Mechanismen der Krebsentstehung und Virusreplikation (HIV), Gentherapie

Institut für Biochemie der FU Berlin
Thielallee 63, D-14195 Berlin, Lise Meitner-Hörsaal
e-mail: moelling@immv.unizh.ch., Tel. 0041-1-634-2652

1. **Signaltransduktion von der Membran zum Kern in normalen Zellen und Tumorzellen (Kinase-Kaskaden)**
2. **Onkogene,Tumorsuppressor-Gene und Telomerase**
3. **Viren bei der Krebsentstehung (HIV, HBV, HPV, EBV, etc.)**
4. **Gentherapie**

Termine

1. **Freitag, 10. November 2000, 15.15 bis 18.00 Uhr.**
2. **Freitag, 8. Dezember 2000, 15.15 bis 18 Uhr. Literatur: (1), (2)**
3. **Freitag, 19. Januar 2001, 15.15 bis 18 Uhr. Literatur: (3), (4)**
4. **Freitag, 9. Februar 2001, 15.15 bis 18.00 Uhr. Literatur: (5), (6)**

Literatur:

(1) S.S. Lee, R.S. Weiss and R.T. Javier: Binding of human virus oncoproteins to hDlg/SAP97, a mammalian homolog of the *Drosophila* discs large tumor suppressor protein. *Proc. Natl. Acad. Sci. USA* **94**, 6670-6675 (1997).
(2) T.-C. He, A.B. Sparks, C. Rago, H. Hermeking, L. Zawel, L.T. da Costa, P.J. Morin, B. Vogelstein, K.W. Kinzler: Identification of c-Myc as a target of the APC pathway. *Science* **281**, 1509-1512 (1998).
(3) W.C. Hahn, Ch.M. Counter, A.S. Lundberg, R.L.Beijersbergen, M.W. Brooks and R.A. Weinberg: Creation of human tumor cells with defined genetic elements. *Nature* **400**, 464-468 (1999).
(4) H. Dong, R.J. O'Brien, E.T. Fung, A.A. Lanahan, P.F. Worley and R.L. Huganir: GRIP: a synaptic PDZ domain-containing protein that interacts with AMPA receptors. *Nature* **386**, 279-284 (1997).
(5) C.P. Hunter: Genetics: A touch of elegance with RNAi. *Current Biology*, **9**, R441-R442 (1999).
(6) P.A. Sharp: RNAi and double-strand RNA. *Genes & Dev.* **13**, 139-141 (1999).

Möglichkeit zum Laborpraktikum für 2 Monate nach Absprache in Zürich vorhanden (Adresse etc. siehe oben) (entspricht 8-12 Sem. Wo. Std.).

INSTITUTSKOLLOQUIUM 2005

Montag, 10., Dienstag, 11., Mittwoch, 12. Januar 2005
(eventuell Donnerstag, 13. Januar)

- Zielsetzung
- Titel
- Einführung mit Abbildungen, Modellen etc (ca. 6 Abb.)
- Experimente mit Daten (beschriftet, mit Methode z.B. als Schema, Legende)
- Neueste Ergebnisse auch als Folie
- Liste der relevanten Publikationen mit update (ca.10)
- Zusammenfassung der Ergebnisse (Liste)
- Fragen, Planung (Liste)
- Timeline (wann, was....)
- Publikationsplanung (Termin, Journal)
- Kritik (Selbstevaluation, Thema)
- Verbesserungsvorschläge

Präsentation mit Hand-outs

VLAK	Th. Fritzius mit J. Heinrich, A. Caelers
Src	G. Radziwill, A. Weiss, J. Heinrich, M. Lorger
HIV	J. Heinrich, A. Ziogas
DNA	J. Pavlovic, L. Elzaouk, N. Usluoglu
Viren	J. Pavlovic, B. Oberle, S. Deuber, E. Ortner
Bcr	A. Ress
other topics:	G. Radziwill, R. Fritz, M. Lorger, A. Ziogas

FINAL CONCLUSION OF INSTITUTSKOLLOQUIUM
(self-evaluation sheet)

Name:
Title of Project:
Priority list:
Main conclusions of discussion:
Time line:
Goal (paper, exam, etc.):
Journal:

TSB TECHNOLOGIESTIFTUNG INNOVATIONSZENTRUM BERLIN

Zukunftsfonds Berlin

Technologie- und Innovationsrat des Landes Berlin (TIR) gleichzeitig Beirat des Kuratoriums der TSB

Der Technologie- und Innovationsrat des Landes Berlin (TIR) wurde im Januar 2000 durch den Regierenden Bürgermeister von Berlin berufen. Er berät das Land Berlin in allen technologiepolitischen Fragestellungen. Der TIR ist gleichzeitig Beirat des Kuratoriums der TSB Technologiestiftung Innovationszentrum Berlin. Auf der Grundlage von Empfehlungen des TIR trifft das Kuratorium der TSB TSB Förderentscheidungen im Zukunftsfonds Berlin.

Die **Mitglieder** des TIR sind:

- **Prof. Dr. Klaus Backhaus**, Direktor des Betriebswirtschaftlichen Instituts für Anlagen- und Systemtechnologie, Westfälische Wilhelms-Universität, Münster
- **Prof. Dr. Eleanor Campbell**, Dept. Of Experimental Physics, Göteborg University & Chalmers, Göteborg / SE
- **Prof. Dr. Manfred Erhardt**, Generalsekretär des Stifterverbandes für die Deutsche Wissenschaft a.D. (Beiratsvorsitzender)
- **Dr. Manfred Gentz**, Vorsitzender des Kuratoriums der TSB
- **Rainer Grohe**, Executive Director, Galileo Joint Undertaking, Brüssel
- **Prof. Jörg Menno Harms**, Geschäftsführer der Menno Harms GmbH, Aufsichtsratsvorsitzender der Hewlett Packard GmbH
- **Prof. Dr. Karl-Heinz Hoffmann**, Direktor der Stiftung caesar
- **Prof. Dr. Georg Friedrich Melchers**, Leiter des Instituts für Immunologie Basel
- **Prof. Dr. Karin Mölling**, Direktorin des Instituts für Medizinische Virologie der Universität Zürich
- **Prof. Dr. Claus Weyrich**, Mitglied des Vorstands, Corporate Technology, Siemens AG, München

Die Mitgliedschaft im TIR ist eine persönliche Mitgliedschaft. Die Mitglieder sind für die Dauer von 5 Jahren berufen.

Kontaktadresse des TIR ist die Geschäftsstelle des Zukunftsfonds.

Zum Zukunftsfonds-Inhaltsverzeichnis Zum Seitenanfang

HELMHOLTZ GEMEINSCHAFT

http://www.helmholtz.de/de/Aktuelles/Helmholtz-Ausschreibungen/Preistraeger_Wissenschaftspreis_des_Stifterverbandes_-_Erwin_Schroedinger_Preis.html

Wissenschaftspreis des Stifterverbandes - Erwin Schrödinger Preis

Abgabetermin für die Vorschläge ist der 1. März des Verleihungsjahres.

Vergeben für interdisziplinäre Forschung durch die Hermann von Helmholtz-Gemeinschaft Deutscher Forschungszentren

Der **Wissenschaftspreis des Stifterverbandes - Erwin Schrödinger Preis** zeichnet herausragende wissenschaftliche oder technisch innovative Leistungen aus, die in Grenzgebieten zwischen verschiedenen Fächern der Medizin, Natur- und Ingenieurwissenschaften erzielt worden sind und an denen Vertreterinnen und Vertreter mindestens zweier Fachrichtungen mitgewirkt haben.

Der **Wissenschaftspreis des Stifterverbandes - Erwin Schrödinger Preis** wurde der Hermann von Helmholtz-Gemeinschaft Deutscher Forschungszentren vom Stifterverband für zunächst 5 Jahre zur Verfügung gestellt. Der Preis ist mit bis zu **50.000 EURO** dotiert. In der Verwendung des Preisgeldes sind die Preisträger frei. Der Preis wird jährlich im Rahmen der Helmholtz-Jahrestagung offiziell übergeben. Die erste Preisverleihung erfolgte am 25. November 1999. Nach Ablauf der fünfjährigen ersten Vergabeperiode hat der Stifterverband beschlossen, den Preis in unveränderter Form weiterzuführen, jedoch von einem einjährigen auf einen zweijährigen Vergabeturnus überzugehen. Die Helmholtz- Mitgliederversammlung hat daraufhin beschlossen in den Zwischenjahren den Preis zu dotieren, so dass im Einverständnis mit dem Stifterverband der Preis weiter jährlich vergeben wird: abwechselnd dotiert vom Stifterverband und der Helmholtz-Gemeinschaft.

Preisträger können (mindestens zwei) Einzelpersonen oder auch Personengruppen sein. Die ausgezeichneten Arbeiten sollen mindestens teilweise in einem Helmholtz-Zentrum entstanden sein. Ihr erfolgreicher Abschluss sollte nicht mehr als 5 Jahre zurückliegen.

Vorschlagsberechtigt sind die Mitgliedseinrichtungen der Helmholtz-Gemeinschaft vertreten durch ihre Vorstände. Es können auch mehrere Vorschläge pro Zentrum abgegeben werde. Die Wiederholung von Vorschlägen ist zulässig. Die Vorschläge sind mit den jeweiligen Wissenschaftlichen Räten der Helmholtz-Zentren abzustimmen.

Die **Anforderungen für die Vorschlagsunterlagen** entnehmen Sie bitte dem PDF "Informationen zum Wissenschaftspreis des Stifterverbandes - Erwin Schrödinger Preis"

Da die Helmholtz-Gemeinschaft im Zuge der Realisierung der Chancengleichheit bestrebt ist, die Sichtbarkeit der Leistungen von Frauen im wissenschaftlichen Bereich zu erhöhen, werden die Vorschlagsberechtigten gebeten, in besonderem Maße geeignete Wissenschaftlerinnen bei ihren Vorschlägen zu berücksichtigen.

Die bisherigen Preisträger des Wissenschaftspreis des Stifterverbandes - Erwin Schrödinger Preis:

HELMHOLTZ GEMEINSCHAFT

Erwin Schrödinger (1887-1961): Der Begründer der Wellenmechanik

Prof. Dr. Karin Moelling is president of the Jury committee for the Schrödinger-Prize (since 1999).

Vorwort Jahrbuch der Helmholtz Gemeinschaft 2004

Research crosses borders: Was bedeutet dies für die größte Wissenschaftsorganisation in Deutschland?

Unser Namenspatron Hermann von Helmholtz hat einmal gesagt: Je mehr der einzelne Forscher gezwungen ist, das Feld seiner Arbeit zu verengen, desto mehr spürt er das Bedürfnis, den Zusammenhang des Ganzen nicht zu verlieren. Wo sonst solle er die Kraft und die Freudigkeit für seine mühsame Arbeit hernehmen? Wenn nicht aus der Überzeugung, dass er einen Baustein geliefert hat zu dem großen Ganzen der Wissenschaft im Dienste der sittlichen Zwecke der Menschheit. Altmodische Formulierungen des 19. Jahrhunderts. Aber der Inhalt stimmt noch immer. Die Helmholtz-Gemeinschaft will Bausteine liefern zur Lösung großer Fragen und drängender Probleme der modernen Gesellschaften. Wir stellen uns den globalen Herausforderungen.

Deshalb müssen wir Grenzen überschreiten: innerhalb unserer Gemeinschaft und darüber hinaus. Helmholtz-Forscherinnen und -Forscher arbeiten in internationalen Teams. Sie kooperieren weltweit in strategischen Allianzen, an denen sich unterschiedliche Institutionen beteiligen und in denen Interdisziplinarität die selbstverständliche Arbeitsform ist. Nur so können wir die Grenzen des Wissens immer weiter verschieben, relevante Erkenntnisfortschritte erzielen und mit unseren Ergebnissen die Brücke zu innovativen Anwendungen schlagen.

In diesem Heft zeigen Beispiele aus der Forschungsarbeit der Helmholtz-Zentren, was „research crosses borders" in der Praxis bedeutet. Ob reibungsloser Bahnverkehr in Europa, Sanierung großer belasteter Gebiete oder Energie aus solarthermischen Kraftwerken, ob Impfstoff gegen Aids, neue Materialien für den Automobilbau oder ein Röntgenlaser, der Filme im Mikrokosmos „drehen" kann. So unterschiedlich und komplex die Themen sind, wir bearbeiten sie mit der gleichen Strategie. Wir bündeln Kräfte – auf nationaler Ebene, in europäischen Kooperationen und großen internationalen Projekten –, um gemeinsam mit unseren Partnern mehr zu erreichen. Aber lesen Sie selbst.

Prof. Dr. Walter Kröll
Präsident der Helmholtz-Gemeinschaft

„Unsere Motivation ist herauszufinden, wie weit wir gehen können. Denn selbst wenn wir Dioden und Transistoren im Nanomaßstab haben, müssen wir noch beweisen, dass sie konkurrenzfähig sind."

Heiko Weber

Die Chemie muss stimmen

Karlsruher Forscherteam erhielt Erwin Schrödinger-Preis.

Erwin Schrödinger-Preis

Grenzen überschreiten – das ist das Thema des Erwin Schrödinger-Preises. Vergeben wird er für interdisziplinäre Forschung. Dieses Jahr zeichnete die Helmholtz-Gemeinschaft ein Forscherteam aus dem Institut für Nanotechnologie des Forschungszentrums Karlsruhe mit dem mit 50.000 Euro dotierten Preis aus. Damit honorierte die Jury das Team aus Physikern und Chemikern für seine exzellenten interdisziplinären Leistungen in der Nanotechnologie.

Zwei bahnbrechende Arbeiten machten die Forscher Dr. Frank Hennrich, Dr. Ralph Krupke, Dr. Marcel Mayor und Dr. Heiko Weber unter Fachkollegen in den letzten Jahren weltweit bekannt: Sie entwickelten ein seit langer Zeit gesuchtes Verfahren zur Trennung von winzigen Kohlenstoffröhrchen, die in der Nanotechnologie eine wichtige Rolle spielen. Und: Es gelang ihnen, den elektrischen Strom durch einzelne organische Moleküle zu vermessen. Durch systematische Zusammenarbeit hat das Karlsruher Team damit zwei grundsätzliche Probleme gelöst, die das gesamte Arbeitsgebiet der Nanotechnologie betreffen. Zusammen ebnen ihre Arbeiten den Weg zu einer künftigen Nanoelektronik, bei der winzige Schaltkreise in der Größe von millionstel Millimetern gebaut werden könnten. Dieser Elektronik im kleinsten Maßstab wird etwa in der Computer-, Satelliten- oder Medizintechnik eine wichtige Rolle vorausgesagt.

„Wir mussten viel miteinander reden und diskutieren. Aber Physiker und Chemiker sprechen zunächst einmal verschiedene Sprachen und benutzen ein unterschiedliches Fachvokabular. Um unsere Kräfte zu bündeln, mussten wir also erst einmal eine gemeinsame Sprache finden. Nur so war eine erfolgreiche Kooperation möglich."

Ralph Krupke

„Makkaroni" aus Kohlenstoffatomen

Bereits 1991 entdeckten japanische Forscher, dass sich Kohlenstoffatome zu winzigen Röhrchen formen können, deren Wände nur eine Atomlage dick sind. Insbesondere in der molekularen Elektronik galten sie seither als Grundbausteine elektronischer Bauteile. Bisher jedoch gab es eine Schwierigkeit: Bei der Herstellung entsteht immer ein Gemisch aus zwei Typen von Nanoröhren mit unterschiedlichen elektrischen Eigenschaften. Je nach Anordnung der Atome in den Wänden der Röhrchen verhalten sich die „Kohlenstoffmakkaroni" entweder wie Metalle oder wie Halbleiter. In der Mischung sind die metallischen und die nichtmetallischen Röhrchen wie ein dichter Filz untereinander verknäult und somit nicht gut nutzbar. Der Physiker Krupke und der Chemiker Hennrich trennten die halbleitenden und metallischen Röhrchen in einer Lösung voneinander und sortierten sie. „Der letzte Schritt war, dass die metallischen und die halbleitenden Nanoröhren in einem elektrischen Wechselfeld mit einer Frequenz von 10 Millionen Hertz in entgegengesetzte Richtungen wandern," erklärt Krupke.

„Die Röhrchen sind Hoffnungsträger für die Computerindustrie: Metallische eignen sich als Leiterbahnen auf Computerchips, und halbleitende könnten aufgrund

pläre Forschung: Alle Disziplinen, von denen man denkt, dass sie für eine zielstrebige Forschung in der Nanotechnologie wichtig sind, vereint das Institut unter einem Dach. Hier herrscht die Arbeitsatmosphäre, die interdisziplinäre Visionen und Projekte fördert."

Marcel Mayor

ihrer physikalischen Eigenschaften als Transistor eingesetzt werden", sagt Hennrich. Für das Trennverfahren erteilte das Europäische Patentamt den beiden Forschern im Herbst 2004 ein Patent.

Die Preisträger von links nach rechts: Dr. Ralph Krupke, Dr. Heiko Weber, Dr. Marcel Mayor und Dr. Frank Hennrich. Professor Karin Mölling (Mitte) ist Vorsitzende der Jury, die die Preisträger auswählt.

Foto: Thierry Monasse

Moleküle unter Strom

„Dort, wo physikalische Bauteile immer kleiner und chemische Moleküle immer größer werden, treffen sich die Physik und die Chemie. An dieser Schnittstelle befindet sich die Nanotechnologie: Schaltungen werden auf molekularer Ebene gebaut. Kleiner geht es nicht mehr!"

Karin Mölling

Für elektrische Schaltungen im Nanomaßstab braucht man aber außer winzigen Drähten weitere Bauteile – zum Beispiel Schalter. Hier bieten sich einzelne Moleküle an. Allerdings muss man sie hierzu elektrisch kontaktieren können. Außerdem benötigt man Moleküle, deren Leitungsmechanismus vorhersagbar ist. Dem Chemiker Marcel Mayor und dem Physiker Heiko Weber gelang hier ein Durchbruch: Sie schafften es, einzelne Moleküle zwischen zwei winzigen Goldelektroden einzuspannen und den Strom durch diese Moleküle zu messen. „Zwar war die Idee, einzelne Moleküle als elektronische Bauteile einzusetzen, nicht neu. Erstmals konnten wir aber die elektronischen Transportprozesse in den Molekülen umfassend vermessen und verstehen. Das bedeutet, dass wir durch die geeignete Wahl der Struktur des Moleküls die elektronischen Eigenschaften der Bauteile tatsächlich festlegen können", erklärt Mayor.

„Damit wir mit unserer Arbeit Erfolg haben, muss mehr funktionieren als die Chemie im Reagenzglas: Auch auf die Chemie unter den Kollegen kommt es an. Wenn die wie bei uns stimmt, kann man sich gegenseitig zu Höchstleistungen anspornen."

Frank Hennrich

Die Erfolge beider Arbeiten überzeugten die Jury: „Den diesjährigen Preisträgern ist auf einzigartige Weise gelungen, interdisziplinär zusammenzuarbeiten und die Bereiche Chemie und Physik auf einem innovativen Forschungsgebiet fruchtbar miteinander zu verbinden", erklärte Professor Karin Mölling, Vorsitzende der Jury und Direktorin des Instituts für Medizinische Virologie an der Universität Zürich.

Dr. Ellen Peerenboom
Kommunikation und Medien
Helmholtz-Gemeinschaft

Synopsis of Clinical Trial

Investigator-driven Phase I Clinical Trial "Immunotherapy in Patients with Metastatic Malignant Melanoma by Intratumoral Injection of Naked Plasmid DNA Encoding Human Interleukin 12" K. Moelling (Scientific coordinator and Sponsor)

1994 - 1999 Preclinical studies with then different cytokine-encoding or tumor-associated antigen-coding DNAs in retroviral vectors or as naked DNA. Various animal models.

- B16-F10 mouse melanoma model and mouse melanoma metastasis in the lung
- Colon cancer, pancreatic cancer, Lewis lung lymphoma, etc.
- Grey horses with naturally occurring melanomas

Publications on preclinical results

Schultz, J., Pavlovic, J., Strack, B., Nawrath, M. and Moelling, K.: Long-lasting anti-metastatic efficiency of IL-12-encoding plasmid DNA. *Human Gene Therapy* **10**, 407-417 (1999).

Schultz, J.,Heinzerling, L. Pavlovic, J. and Moelling, K.: Induction of long-lasting cytokine cascade by injection of IL-12 encoding plasmid DNA. *Cancer Gene Therapy*, **7**, 1557-1565 (2000).

Heinzerling, L., Feige, K., Rieder, S., Akens, M., Dummer, R., Stranzinger, G., Moelling, K.,: Tumor regression induced by intratumoral injection of DNA coding for human interleukin 12 into melanoma metastases in gray horses. *Journal of Molecular Medicine,* **78**, 692-702 (2001).

Publications on clinical results

Heinzerling, L., Burg, G., Dummer, R., Maier, T., Oberholzer, P.A., Schultz, J., Elzaouk, L., Pavlovic, J. and Moelling K.: Intratumoral injection of DNA encoding human interleukin 12 into patients with metastatic melanoma: Clinical efficacy. *Human Gene Therapy* 16, 35-48 (2005).

	Design of Clinical Protocol, Investigator's Brochure, Patient's consent
	1st SKBS meeting: Presentation of preclinical results in mice. Request by SKBS for more results.
18.6.1999	Written approval for human use of IL-12 DNA by Hoffmann-La Roche (after 2 years of negotiation)
24.11.1999	"Pre-IND" meeting organized by K. Moelling with BERNA and BAG, Bern (Glueck, Zurbriggen, Paroz, Struck, Lambert, Pavlovic, Moelling), no criticism on protocol, no decision on production.
Dec. 1999	1st DNA production ("in the spirit of GMP", US and UK standard) supported by KTI project.
3.3.2000	Meeting with Prof. G. Burg, USZ, Dept. of Dermatology, agreement as clinical investigator.
14.3.2000	2nd presentation to SKBS, Bern. Reject of 1st GMP production and request for destruction.
29.3.2000	Approval of clinical protocol Ref. No. GT-2000011.
26.5.2000	Application to Kantonale Ethics Committee (KEK), No. 383
13.7.2000	Approval by Ethics Committee
11.7.2000	Clinical study notification, Bern, and approval
Aug. 2000	2nd GMP-DNA Production by BERNA Co., Bern
Oct. 2000	Approval by BAG for GMP-DNA production 100 mg and tox. study
16.10.2000	Financing of GMP-DNA production by SNF, NFP37
Dec. 2000	Begin of clinical study
Sept.. 2002	Treatment last (9th) patient
Febr. 2002	Compassionate trial Charité, UKRV, Berlin
Nov. 2003	Final report to Swissmedic on the trial with positive evaluation
Jan. 2005	Publication of clinical results in Human Gene Therapy (see above)
2005	Compassionate trial at USZ, Zurich
2005/2006	Continuation of clinical trial

Development of a DNA Vaccine against HIV-1

1990-1993	**Initiation and research on DNA vaccine against HIV-1** Joint-appointment of: Karin Moelling, Head of Research Group at MPI for Molecular Genetics, Berlin, D & Director of Molecular and Cellular Biology, Apollon Inc, USA Responsibility: Development of a clinical approval plasmid DNA construct in collaboration with the FDA, Bethesda, MD, USA
December 1993	**Meeting at the Institute of Medical Virology** Presentation of the project by the CEO of Apollon Inc., Dr. V. Zurawski First evaluation for the possibility of a Swiss trial Invited guests: Prof. Dr. C. Weissmann, Prof. Dr. H. Diggelmann, Head of the SKBS, Prof. Dr. R. Lüthy, USZ
May 1994	**Scientific presentation on DNA vaccine against HIV-1** by Dr. D. Weiner, University of Pennsylvania, Philadelphia, USA
May 1995	**Approval of a phase I clinical trial by the FDA, Bethesda, USA**
June 1995	**First volunteer immunized in Philadelphia, USA**
September 1995	**Approval of the Swiss trial by the SKBS** Scientific coordinator: Prof. Dr. K. Moelling Clinical investigator: Prof. Dr. R. Lüthy
March 1996	**Four volunteers immunized at the University Hospital in Zurich**
March 1997	**Termination of Phase I/II trial in Zürich and Philadelphia**
1998/2002	**Follow-up of patients in Zurich for safety parameters**

Publication: Weber R, Bossart W, Cone R, Luethy R, and Moelling K. Phase I clinical trial with HIV-1 gp160 plasmid vaccine in HIV-1-infected asymptomatic subjects (2001). *Eur. J. Clin. Microbiol. Infect. Dis. 20, 800-803*

Evaluations by extern evaluation organizations

Lecture Virology University

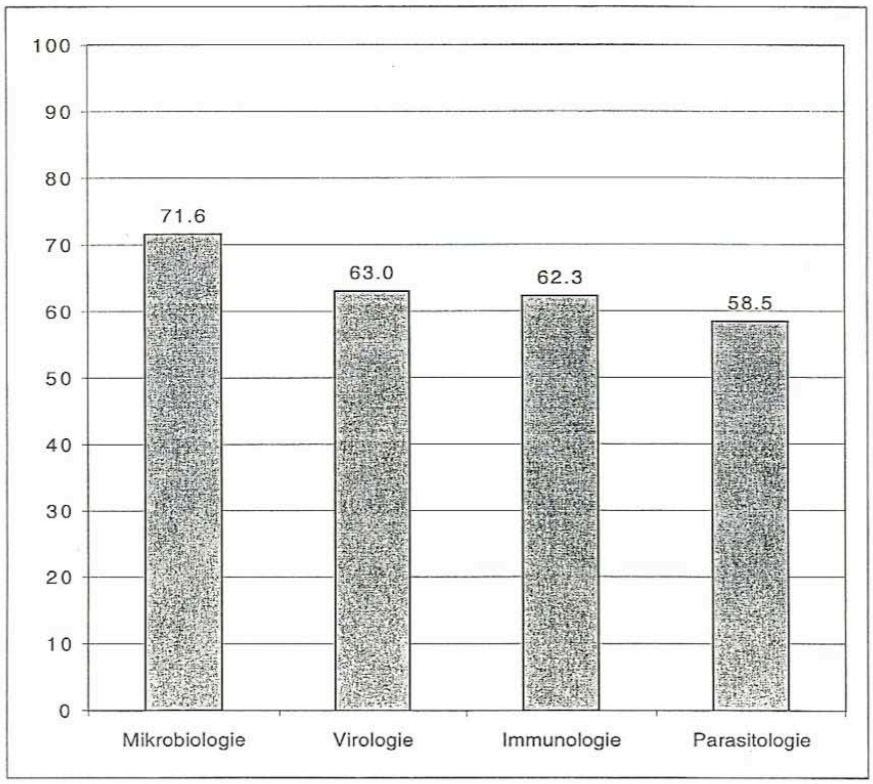
Mikrobiologie Microbiologie

Zürich, 15. Juli 2004

Subscore Liste nach Inhalt

erzielte Leistung

BP	Total Anzahl Items	A,B,E,K Items	Kprim Items	pN Items	Ø[%]
Mikrobiologie	45	33	12	0	71.6
Virologie	20	16	4	0	63.0
Immunologie	21	17	4	0	62.3
Parasitologie	10	8	2	0	58.5
Total	96				



Erstellt durch das IML (Institut für Medizinische Lehre),
Medizinische Fakultät, Universität Bern,
Abteilung für Ausbildungs- und Examensforschung

19.7.2004, aae/iml, rh

ETH

Eidgenössische Technische Hochschule Zürich
Swiss Federal Institute of Technology Zurich

Unterrichtsbeurteilung durch die Studierenden

Einzelauswertung SS04

Gemäss Weisung des Rektors 30.09.2003

Angaben zur Lehrveranstaltung:

Studiengang/Frageset:	**BIOLV-MOLV1**	LV-ID/Dozierende(r):	**551-1134-00 003**
Datum der Erhebung:	**07.06.2004**	Anzahl Formulare:	**18**
Studierende (Immatrik.):	**Alle**		
Name d. Dozierenden:	-		
Bez. Lehrveranstaltung:	-		

Persönliche Fragen an die Studierenden:

Immatrikuliert am Studiengang *):

Anzahl		
100%	17	BIOL

Anzahl

Anzahl

***) Allfällige Ergänzung:**

Anzahl		
11%	2	Hörer/in Nachdiplom.
6%	1	and. Lehran. Übrige

Muttersprache:

Anzahl	
16	Deutsch
	Französisch
	Italienisch
	Rätoromanis.
1	Englisch
	andere

Geschlecht:

7	weiblich
8	männlich

Es gilt, falls nicht anders angegeben:

Die Aussage ... trifft zu:
k.Ä.: keine Äusserung
1: überhaupt nicht
2: in geringem Masse
3: teils, teils
4: grösstenteils
5: in höchstem Masse

Zu den nachfolgenden Daten und Diagrammen:

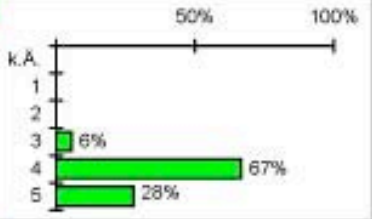
Werte und Diagramme: Diese repräsentieren die Verteilung der auswertbaren Antworten.
Mittelwerte (MW) und Standardabweichungen (SA): Diese werden aufgrund der gültigen Antworten gebildet (ohne k.Ä.).
Prozentwerte: Diese sind berechnet auf der Basis der gültigen Antworten (:=100%)

R1 Frage des Rektors / der Rektorin

Der Dozent/die Dozentin bot einen engagierten Unterricht.

		Anz.
k.Ä.		0
1		0
2		0
3	6%	1
4	67%	12
5	28%	5

MW = 4.2 SA = 0.5 ungültig 0 gültig 18

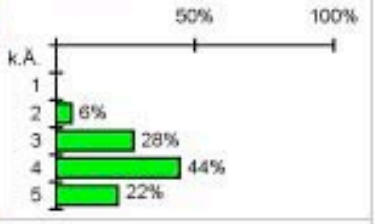


R2 Frage des Rektors / der Rektorin

Der Dozent/die Dozentin vermochte den Stoff verständlich und anschaulich zu erklären.

		Anz.
k.Ä.		0
1		0
2	6%	1
3	28%	5
4	44%	8
5	22%	4

MW = 3.8 SA = 0.8 ungültig 0 gültig 18

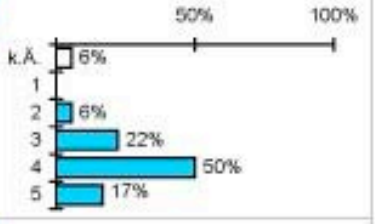


S1 Frage des Studiengangs BIOLV-MOLV1

Der Dozent/die Dozentin vermochte den Studierenden die Bedeutung der Lehrveranstaltung für den Studiengang zu vermitteln.

		Anz.
k.Ä.	6%	1
1		0
2	6%	1
3	22%	4
4	50%	9
5	17%	3

MW = 3.8 SA = 0.8 ungültig 0 gültig 18

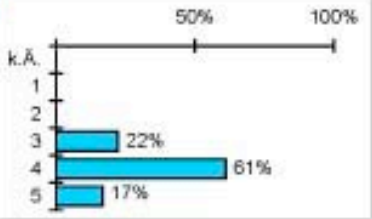


S2 Frage des Studiengangs BIOLV-MOLV1

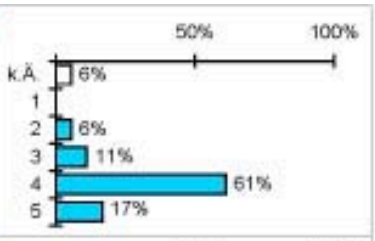
Die Vorlesung war gut strukturiert (Aufbau, Transparenz, roter Faden).

		Anz.
k.Ä.		0
1		0
2		0
3	22%	4
4	61%	11
5	17%	3

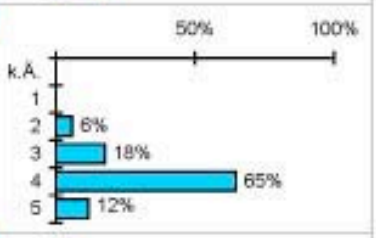
MW = 3.9 SA = 0.6 ungültig 0 gültig 18



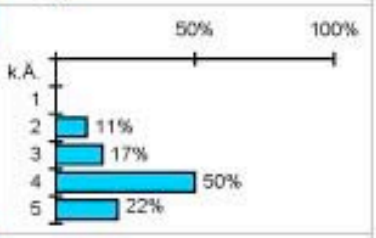
S3	Frage des Studiengangs BIOLV-MOLV1				Anz.
	Die Vorlesung war frei von unnötigen inhaltlichen Überschneidungen mit anderen Lehrveranstaltungen. (Allfällige Überschneidungen bitte im Kommentarfeld angeben!)	k.Ä.	6%	1
		1		0
		2	6%	1
		3	11%	2
		4	61%	11
		5	17%	3
	MW = 3.9 SA = 0.7 ungültig 0		gültig	18



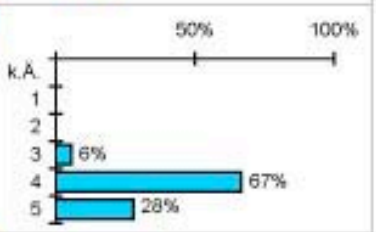
S4	Frage des Studiengangs BIOLV-MOLV1				Anz.
	Die verteilte oder empfohlene Dokumentation war hilfreich (Skript, Handouts, empfohlene Lehrbücher, etc.).	k.Ä.		0
		1		0
		2	6%	1
		3	18%	3
		4	65%	11
		5	12%	2
	MW = 3.8 SA = 0.7 ungültig 1		gültig	17



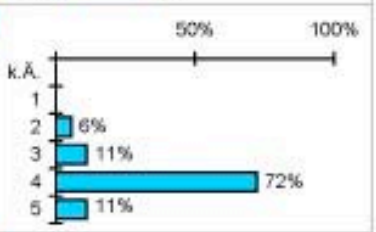
S5	Frage des Studiengangs BIOLV-MOLV1				Anz.
	Es gelang dem Dozenten/der Dozentin die Vorlesung so zu halten, dass man ihr gedanklich folgen konnte.	k.Ä.		0
		1		0
		2	11%	2
		3	17%	3
		4	50%	9
		5	22%	4
	MW = 3.8 SA = 0.9 ungültig 0		gültig	18



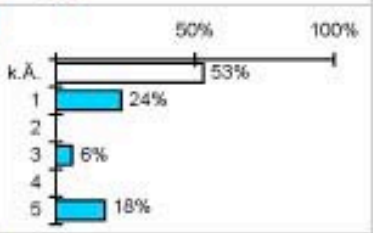
S6	Frage des Studiengangs BIOLV-MOLV1				Anz.
	Der Dozent/die Dozentin ging hilfsbereit auf Fragen und Bemerkungen ein.	k.Ä.		0
		1		0
		2		0
		3	6%	1
		4	67%	12
		5	28%	5
	MW = 4.2 SA = 0.5 ungültig 0		gültig	18



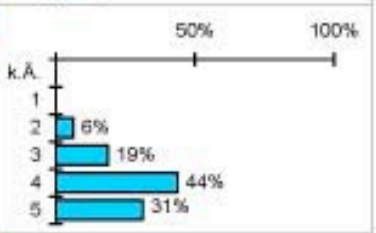
S7	Frage des Studiengangs BIOLV-MOLV1				Anz.
	Der Dozent/die Dozentin setzte die visuellen Hilfsmittel (Wandtafel, Overhead, Beamer, etc.) sinnvoll ein.	k.Ä.		0
		1		0
		2	6%	1
		3	11%	2
		4	72%	13
		5	11%	2
	MW = 3.9 SA = 0.7 ungültig 0		gültig	18



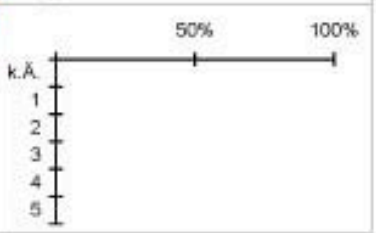
S8	Frage des Studiengangs BIOLV-MOLV1				Anz.
	Der Dozent/die Dozentin hatte den Prüfungsstoff/den Stoff der Leistungskontrolle bekannt gegeben. (1: Nein // 5: Ja)	k.Ä.	53%	9
		1	24%	4
		2		0
		3	6%	1
		4		0
		5	18%	3
	MW = 2.8 SA = 1.9 ungültig 1		gültig	17



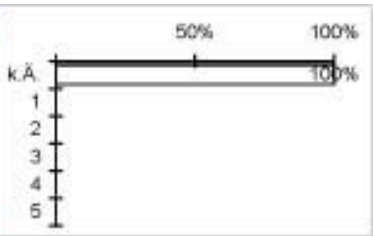
S9	Frage des Studiengangs BIOLV-MOLV1				Anz.
	Nur für Lehrveranstaltungen mit mehreren Dozierenden: Die Dozierenden hatten den zu behandelnden Stoff gegenseitig abgestimmt.	k.Ä.		0
		1		0
		2	6%	1
		3	19%	3
		4	44%	7
		5	31%	5
	MW = 4.0 SA = 0.9 ungültig 2		gültig	16



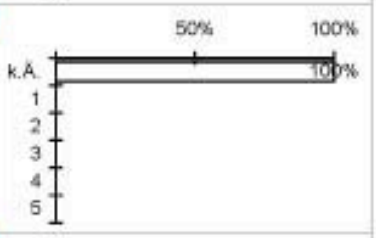
S10	Frage des Studiengangs BIOLV-MOLV1				Anz.
	Die Übungen trugen viel zum Verständnis der Vorlesung bei. (Ausnahmen bitte im Kommentarfeld angeben!)	k.Ä.	#####		0
		1	#####		0
		2	#####		0
		3	#####		0
		4	#####		0
		5	#####		0
	MW = #### SA = #### ungültig 18		gültig		0



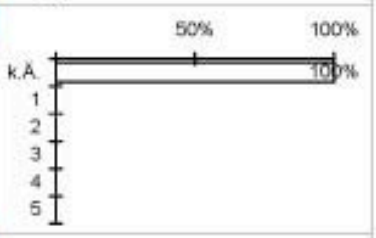
S11	Frage des Studiengangs BIOLV-MOLV1				Anz.
	Die Übungen waren: 1: zu schwierig,	k.Ä.	100%		1
	2: schwierig, 3: gerade richtig,	1			0
	4: einfach, 5: zu einfach.	2			0
	(Zu einfache und zu schwierige Aufga-	3			0
	ben bitte im Kommentarfeld angeben!)	4			0
		5			0
	MW = #### SA = #### ungültig 17			gültig	1



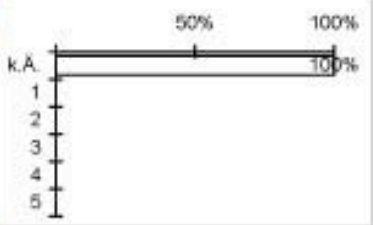
S12	Frage des Studiengangs BIOLV-MOLV1				Anz.
	Für die Übungen benötigte ich zusätz-	k.Ä.	100%		1
	lich zur Präsenzzeit einen wöchent-	1			0
	lichen Zeitaufwand von durchschnittlich:	2			0
	1: 0h // 2: 2h // 3: 4h // 4: 6h //	3			0
	5: >6h	4			0
		5			0
	MW = #### SA = #### ungültig 17			gültig	1



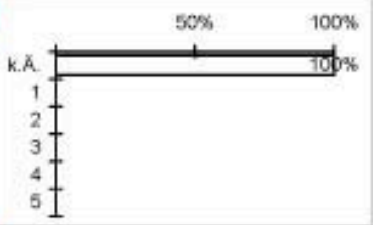
S13	Frage des Studiengangs BIOLV-MOLV1				Anz.
	Es wurden genügend betreuende	k.Ä.	100%		1
	Personen eingesetzt.	1			0
		2			0
		3			0
		4			0
		5			0
	MW = #### SA = #### ungültig 17			gültig	1



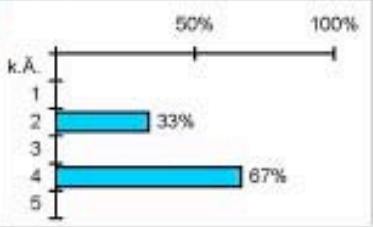
S14	Frage des Studiengangs BIOLV-MOLV1				Anz.
	Die Betreuungspersonen waren	k.Ä.	100%		1
	fachlich kompetent.	1			0
	(Falls Betreuende positiv oder negativ	2			0
	aufgefallen sind, bitte im Kommentarfeld	3			0
	mit Namen und kurzer Begründung	4			0
	angeben.)	5			0
	MW = #### SA = #### ungültig 17			gültig	1



S15	Frage des Studiengangs BIOLV-MOLV1				Anz.
	Die Betreuungspersonen waren	k.Ä.	100%		1
	hilfsbereit.	1			0
	(Falls Betreuende positiv oder negativ	2			0
	aufgefallen sind, bitte im Kommentarfeld	3			0
	mit Namen und kurzer Begründung	4			0
	angeben.)	5			0
	MW = #### SA = #### ungültig 17			gültig	1



S16	Frage des Studiengangs BIOLV-MOLV1				Anz.
	Der Fragebogen erlaubt es mir, mein	k.Ä.			0
	Urteil über diese Lehrveranstaltung	1			0
	hinreichend genau und umfassend	2	33%		1
	auszudrücken.	3			0
		4	67%		2
		5			0
	MW = 3.3 SA = 0.9 ungültig 15			gültig	3



PG Kurs Virologie 2004

2.-3.11. 2004

Kursgesamtbeurteilung

Gut aufgebauter Kurs: Zweistündige Einführung in ein neues Themengebiet. Die Dozenten haben dabei auch das Fundament für das später folgende Paper gegeben. Vorstellung des Papers durch jemanden von uns in Form einer Power Point Präsentation. Abschliessen des jeweiligen Stoffgebietes nach intensiver Diskussion im Plenum. Es wurden gute Handouts verteilt.

Erster Tag

Retroviren, Onkogene, Tumorsuppressoren, Signaltransduktion, HIV
Gut strukturierter Aufbau. Zu Beginn eines neuen Gebietes Erinnerung fundamentaler Kenntnisse wie structure and life cycle of retroviruses, später Erarbeiten der Details mit Hilfe von übersichtlichen Folien. Exzellente Darstellung der Funktionen der Produkte der Protoonkogenen.

Zweiter Tag

Virus Rezeptoren, Molekulare Virus Diagnostik, Virus Vektoren für Gentherapie, siRNA Technologie
Kennenlernen eines interessanten und vielversprechenden neuen Vektors. Der Lentiviral Vector. Vor- und Nachteile wurden klar dargestellt. Vermittlung eines guten Ueberblicks der aktuellen gentherapeutischen Ansätze.

Kurszusammenfassung

Gelungene Einführungen in die verschiedenen Stoffgebiete. Die Abgabe der Handouts hat uns das Folgen des Unterrichts sehr erleichtert. Das aktive Vortragen eines Papers hat uns zusätzlich gefordert. Die abschliessende Diskussion war dazu da, die letzten Unklarheiten aus dem Wege zu räumen. Wir schätzten auch sehr, dass PD Dr. Pavlovic uns am Ende des Kurses eine Rückmeldung gab, wie sein Team uns in diesen beiden Tagen erlebt hat.

Herzlichen Dank an dieser Stelle an Herrn PD Dr. Jovan Pavlovic und seinem ganzen Team für die hervorragende Organisation und Durchführung dieser interessanter Tage!

André von Büren
Barbara Willi

Accrediation of Diagnostics

m e t a s

metrologie und akkreditierung schweiz

akkreditierung

Gestützt auf die Akkreditierungs- und Bezeichnungsverordnung vom 17. Juni 1996 (Stand am 9. Dezember 2003) und die Stellungnahme der eidgenössischen Akkreditierungskommission erteilt die Schweizerische Akkreditierungsstelle (SAS) dem

Institut für Medizinische Virologie der Universität Zürich
Diagnostik
Gloriastrasse 30
CH-8006 Zürich

die Akkreditierung als

Prüfstelle für Diagnostik von humanpathogenen Viren, Viruskrankheiten und ausgewählten Mikroorganismen

nach der Norm ISO/IEC 17025. Der Geltungsbereich ist im offiziellen Verzeichnis akkreditierter Prüfstellen festgelegt.

Akkreditierungszeichen und -nummer: SWISS TESTING STS 263

Datum der Akkreditierung: 3. Juli 2000

Datum der letzten Erneuerung der Akkreditierung: 3. Juli 2005

Gültigkeit der Akkreditierung bis: 2. Juli 2010

CH-3003 Bern-Wabern, 23. Juni 2005

Schweizerische Akkreditierungsstelle

Der Leiter

Hanspeter Ischi

Die SAS ist Mitglied der multilateralen Abkommen der European co-operation for Accreditation (EA) für die Bereiche Kalibrieren, Prüfen, Inspizieren und Zertifizieren von Produkten, Personal, Qualitäts- und Umweltmanagementsystemen, des International Accreditation Forum (IAF) für die Bereiche Zertifizieren von Produkten, Qualitäts- und Umweltmanagementsystemen und der International Laboratory Accreditation Cooperation (ILAC) für die Bereiche Kalibrieren und Prüfen.

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Eidg. Justiz- und Polizeidepartement
Département fédéral de justice et police
Dipartimento federale di giustizia e polizia
Swiss Federal Department of Justice and Police

Some critical thoughts

Exit Strategy for Postdocs:

Conventional thinking of postdocs for their future profession is the hope to get a permanent position in research institutions. Such positions become very rare. I tried to encourage my postdocs to consider other possibilities. The two most intelligent postdocs continued to study business and administration and joined the UBS bank. Another postdoc entered an additional school for diplomats to join the EU. I am pleased to see that several of the postdocs were highly accepted by colleagues from the Medical Faculty because my coworkers have a very thorough training in molecular biology, which is rare at the Medical faculty but standard at the MN Faculty. Several of the postdocs went on for postdoc training in the United States.

However, I considered it as important that alternative professional possibilities for postdocs should be emphasised in a more general way by the University. Several of the offers I made for the students were considered as too non-conventional and therefore not accepted. One such possibility is very fashionably designated as translational research, meaning a close collaboration between basic research and the medical field. Especially in this respect I made very serious efforts. Based on my own preclinical data with a gene therapy against cancer I went through the whole procedure to put these data into practice. I managed to find the money to pay for the GMP material, and identified a company for its production. I had to get the permission from Hoffmann la Roche, who gave us the plasmid DNA, which was very difficult. With a coworker I designed and wrote the clinical protocol, got approval by the Swiss Authorities, the Ethics Committee, identified the clinical investigators, conceived the regimen of treatment of the patients, evaluated the results because we could not afford a clinical research organization (CRO), and finally wrote the publication and report to the leading authorities. We filed a patent, which was issued in 2005. Many obstacles hampered this whole procedure. Instead of giving my coworkers future perspectives, it had the opposite effect, to stay out. My own investment of time and energy was not worth it.

Another possibility could have been for my coworkers to invest time into biotechnology e.g. for start-up companies. The research would have allowed several possibilities but did not encounter interest. Biotech is unattractive since several years.

In a very non-conventional way I employed a Business Developer for 1 1/2 years who was a very cooperative stimulating colleague with some different approaches to the non-academic world of business, however also this was not imitated, and not attractive enough for this coworker who hoped for a start-up company. This did not materialize till today.

As far as legal questions are concerned we are encouraged to write patent applications for research results, which might lead to useful applications. Several of these have been written and submitted by myself and coworkers, one patent was issued. Here my experience is that coworkers who are involved in the patent application as co-inventors are not capable or not willing to contribute the necessary workload involved in the following years. In fact most co-inventors have left the institute long before the examiners from the patent offices or the interaction with the patent attorneys require intellectual input. As a consequence everything is left to the Head of the Institute.

I suggest that this should be more openly discussed so that inventors who apply for a patent learn what kind of efforts are involved. The University transfer office, Unitectra, is very cooperative but cannot take over actions, which require especially scientific knowledge of the inventors. I miss the help for partnering with interested investors or cooperators. There is no help with partnering to industry.

Furthermore, the amount of money involved is an aspect, which also should be discussed in more details including from science organisations such as SNF or DFG who encourage the scientists to submit patent applications. The time and money involved is unrealistic. Applications are out of reach, as described above.

A very serious drawback for putting scientific results into practice is not only by the lack of funds but also regulatory. There is no grant organization that is willing or able to support clinical trials. By a law from the EU it is forbidden to perform clinical studies, which might potentially lead to conflicts with the pharmaceutical industry. Here for many years Biotech Companies filled a gap, however, the market was so hype that a complete reversion has taken place and almost no venture capital (VC) money can be raised presently.

It maybe worth mentioning that in the year 2004 350 clinical trials were performed in England, 30 in Switzerland, and 17 in Germany. This indicates that Switzerland is doing quite well, that Swissmedic was e.g. in my case very supportive but some other sources of funding are certainly required. Maybe the German structure of KKS (Kommission für Klinische Studien) Institutions is worth copying. About 20 Universities in Germany installed such a group. This was organized and financed by the BMBF (Bundesministerium für Bildung und Forschung) but did not function in a professional way yet at the Charité.

Another suggestion would be that the technology transfer office (Unitectra) of the University should be given the capacity to help in partnering of the university with industry and commercialization. The equivalent structure at the Max-Planck society has to fulfil this obligation. In the case of return of investment the University will get 50 %. This could be a source for the financing of partnering. Statistically only one out of 100 candidates will succeed at the market, meaning that prefinancing may be required.

It is very unfortunate that the Medical Faculty is so often in the press and in public media because of misconduct or problems with new appointments (Berufungszusagen). This has consequences for the public image as well as the recruitment of patients and on our diagnostics and mechanisms should be developed for internal procedures to avoid the causes, e.g. appointments of professors, unclear promises and unclear structures, which have caused trouble in many cases throughout the last years, including for me.

The crisis affected my research, because an unclear situation at the Department of Dermatology had the consequence that four grant proposals, which I submitted during the last two years, were not processed as long as the controversy about the Department was not settled, in spite of the fact, that I was not involved.

Personal aspects

I was appointed in 1993 as Head of the Institute of Medical Virology. My international reputation in those days was based on my HIV research. I was promised in written that I would be in charge of the National Center of Retrovirology (NZR), which was going to be integrated into my Institute. This was the reason for me to give up a permanent group-leader position at the Max-Planck-Institute in Berlin (C3 Professorship) and to come to Zurich. Within a few months it become transparent that the promised HIV Center would not become part of my Institute. I had been told in written that the Head of Bundesamt für Gesundheit (BAG) had agreed on the integration of the NZR into the IMV under my Directorship. The University cancelled unilaterally a Regierungsratbeschluss. A decision was made, that the NZR was going to be independent but part of my high containment laboratories by a “theoretical border through the hallway” and that I had to supply the infrastructure. I was told that within 5 years new laboratories would become available for the NZR at the Irchel. This was not the case. I never could get access to the HIV center and was also cut off from HIV grants even from the SNF probably by political actions without my official knowledge. HIV diagnostics is not part of the IMV.

When I came to Zurich in 1993 I was told that the renewal of my contract after 5 years was a routine act. This was not the case. At the end of 1997 the University set up an “investigation”, which was initiated by the Prorector with the goal not to renew my contract. I was forced by the Rector to hire a lawyer. He found an unexpected letter dating back to 1993, two weeks before I started my work in Zurich, in which the same Prorector had written that he integrated the NZR into the Department of Veterinary Virology, his own department not into my Institute, but

that the newly appointed director (me) of the IMV should not know about this and that nobody in the University was allowed to tell me.

In 1998 I received a letter about a provisional employment until the end of 1999. Since that day I was not officially employed and was without contract till 2005. Indeed a recommendation to the University Council (Rat) concerning my contract renewal was postponed repeatedly. In a letter in March 2005 in context with this evaluation I was informed to be officially employed from now on based on the argument that I did not misbehave. This indicates that I had a conditional position and was under observation for 6 years!

The reason why I mention this in this evaluation is two-fold:

I performed very little research on viruses because I lost my field of expertise (HIV) and could not raise funds, and turned to some other viruses with less intensity and cancer viruses and viral oncogenes instead.

The amount of energy and thinking and development of survival strategies for my scientific future and my personal life severely reduced the quality of my life and of my research as well as my scientific output.

Karin Moelling

Zurich, December 2005

List of Documents displayed on the desk for more information

- Clinical Protocol (old)
- Clinical Protocol (new)
- Lecture “Virology” Uni Zürich
- Lecture “Studiengang Mikrobiologie” Uni/ETH
- Lecture FU-Berlin
- Annual Reports (1999 – 2004)
- Annual Report 2005