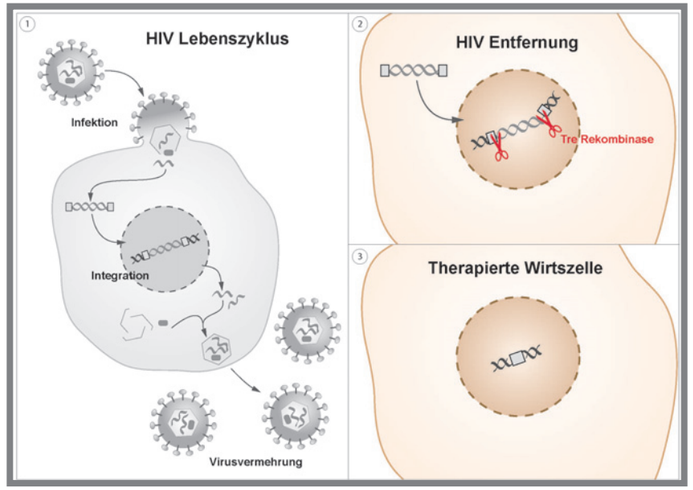
Forschungsbericht

2007



HPI Forschungsbericht 2007

HPI

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Leibniz
Gemeinschaft

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Konzept der Rekombinase-vermittelten HIV-Therapie siehe Abstract auf Seite 25 für Details

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Vorwort

Der vorliegende Forschungsbericht informiert in kompakter Form über Forschungsprogramme und wissenschaftliche Aktivitäten des Heinrich-Pette-Instituts (HPI) im Jahre 2007. Er ist als ein „Update“ des ausführlicheren und bebilderten Tätigkeitsberichts 2005/2006, der im Februar 2007 erschien, zu verstehen, fasst wichtige neue Forschungsergebnisse zusammen und stellt aktuelle neue Entwicklungen aus dem Jahre 2007 vor. Damit soll dieser Bericht interessierten Kollegen, unseren Zuwendungsgebern und nicht zuletzt auch einer breiteren Öffentlichkeit vermitteln, welche Fragestellungen uns im Rahmen der von uns betriebenen wissenschaftlichen Grundlagenforschung beschäftigt haben und welche Fortschritte wir im Jahr 2007 erzielen konnten.

Das HPI ist nun schon seit vielen Jahren eine der ersten Adressen der virologischen und tumorbiologischen Forschung nicht nur am Wissenschaftsstandort Hamburg, sondern auch deutschlandweit. Diese Exzellenz hat der Wissenschaftliche Beirat des HPI in seiner Begehung 2007 dem HPI erneut bestätigt. Wir freuen uns über diese Anerkennung. Sie ist Ansporn, unsere anwendungsnahe Grundlagenforschung auf höchstem Niveau weiter zu betreiben und auszubauen.

Wir sind zuversichtlich, dass die im vorliegenden Bericht dokumentierte Leistungsbilanz des vergangenen Jahres unsere Zuwendungsgeber, die Mitglieder des Kuratoriums und des Wissenschaftlichen Beirats, Freunde und Förderer des HPI und auch die Öffentlichkeit von der wissenschaftlichen und gesellschaftlichen Bedeutung der Forschungsarbeit des Heinrich-Pette-Instituts überzeugen wird.

Hamburg, im Januar 2008

Prof. Dr. Wolfgang Deppert
Vorsitzender des Kollegiums

Wissenschaftliche Highlights

Nur selten werden in den Nachrichtensendungen großer deutscher Fernsehsender neue wissenschaftliche Ergebnisse der breiten Öffentlichkeit vorgestellt. Umso erfreulicher für das HPI war es deshalb, dass die Ergebnisse einer Kooperation von Prof. Dr. Joachim Hauber und Dr. Ilona Hauber (HPI) mit der Arbeitsgruppe um Dr. Frank Buchholz (Max-Planck-Institut für Molekulare Zellbiologie und Genetik) den Weg in diese Medien gefunden haben. Den Wissenschaftlern gelang es, mit Hilfe eines spezifisch konstruierten „Design"-Enzyms das HI-Provirus, welches in das zelluläre Genom integriert ist, auszuschneiden und auf diese Weise die Zelle HIV-frei zu machen. Die Ergebnisse wecken große Hoffnungen, dass es eines Tages möglich sein wird, AIDS durch Viruseliminierung zu heilen – bisher kann man diese Krankheit nur mehr oder weniger gut durch Blockierung der Virusvermehrung in Schach halten. Sicherlich ist das noch Zukunftsmusik. Aber immerhin wurde das von Prof. Hauber eingereichte Projekt „Eradikation proviraler HIV-1 DNA aus Patientenzellen" Sieger im GO-Bio Wettbewerb des Bundesministeriums für Bildung und Forschung. Die mit diesem Preis verbundenen nicht unerheblichen Geldmittel erlauben nun eine Überprüfung der Machbarkeit.

Promotionspreise sind oft die ersten sichtbaren Zeichen einer beginnenden Wissenschaftlerkarriere. Wir freuen uns deshalb, dass Dr. Heike Helmbold aus der Abteilung „Tumorvirologie" des HPI den Promotionspreis 2007 des Fachbereichs Chemie der Fakultät für Naturwissenschaften der Universität Hamburg gewonnen hat. In ihrer Doktorarbeit untersuchte Heike Helmbold das Phänomen der zellulären Seneszenz in Tumorzellen, eines neu entdeckten Schutzmechanismus, der das Wachstum von Tumorvorstufen blockiert und somit deren Auswachsen verhindert.

Den vom Freundes- und Förderkreis des Universitätsklinikums Hamburg-Eppendorf gestifteten Jürgen Lüthje-Promotionspreis 2007 hat Dr. med. Gabriele Iwanski erhalten. Gabriele Iwanski hat ihre Doktorarbeit in der Arbeitsgruppe „Molekulare Pathologie" des HPI durchgeführt und arbeitet jetzt als Assistenzärztin in der Onkologie und Hämatologie des Universitätsklinikums Hamburg-Eppendorf. Den beiden Preisträgerinnen sei an dieser Stelle nochmals herzlich gratuliert!

Für besondere wissenschaftliche Leistungen junger Mitarbeiter hat das HPI einen Nachwuchspreis ausgelobt, der im Jahr 2007 zum ersten Mal verliehen wurde und von nun an jährlich vergeben werden soll. Die ersten Preise erhielten Barbara Fries aus der Abteilung „Zellbiologie und Virologie" für ihre im *Journal of Biological Chemistry* veröffentlichte Arbeit "Analysis of Nucleocytoplasmic Trafficking of the HuR Ligand APRIL and its Influence on CD83 Expression", sowie Heike Helmbold für ihre in *Oncogene* erschienene Publikation „Regulation of Cellular Senescence by Rb2/p130". Den Preisträgern einen herzlichen Glückwunsch und viel Erfolg bei ihren weiteren Arbeiten!

Neue Nachwuchsgruppe

Seit Dezember 2007 hat das HPI eine neue Nachwuchsgruppe „Virus-Pathogenese". Sie wird von Dr. Michael Schindler geleitet. Michael Schindler ist ein schon mehrfach ausgezeichneter Jungforscher, den das HPI von der Universität Ulm nach Hamburg holen konnte. Die

Einrichtung dieser Nachwuchsgruppe wurde durch Fördergelder aus dem Wettbewerbsverfahren „Pakt für Innovation und Forschung" des BMBF ermöglicht. Wir wünschen Michael Schindler gutes Eingewöhnen in Hamburg und im HPI und viel Erfolg in seiner Forschung.

Veranstaltungen

Die beiden wissenschaftlichen Programmbereiche des HPI – „Zelluläre Dysregulation" und „Virus-Wirts-Wechselwirkungen – haben jeweils auf einem Seminartag ihre Projekte vorgestellt und diskutiert („Zelluläre Dysregulation" am 29. März 2007; „Virus-Wirts-Wechselwirkungen" am 7. Dezember 2007). Dabei wurden die Projekte vorwiegend von jüngeren Mitarbeitern präsentiert. Die beiden Seminartage waren ein großer Erfolg, da sie nicht nur einen umfassenden Überblick über laufende Forschungsarbeiten des HPI gewährten, sondern auch wertvolle Anregungen für weitere Kooperationen innerhalb des HPI gaben.

Im Rahmen des Seminartags zum Programmbereich „Virus-Wirts-Wechselwirkungen" am 7. Dezember 2007 wurden nicht nur die oben erwähnten Nachwuchspreise verliehen, sondern auch neu geschaffene Mobilitätsstipendien von bis zu € 5.000 vergeben. Diese Stipendien sollen einerseits jungen HPI-Wissenschaftlern/innen ermöglichen, begrenzte Forschungsaufgaben im Ausland durchzuführen, und andererseits jungen Forschern aus dem Ausland die Möglichkeit geben, für begrenzte Zeit am HPI zu arbeiten. Über die Verleihung eines solchen Stipendiums können sich freuen: Birte Niebuhr, Sabrina Schreiner und Mouna Mhamdi. Weiterhin wird Anna Brestovitsky, Doktorandin am Dept. Of Molecular Microbiology, Technion, Israel, zu einem Forschungsaufenthalt an das HPI kommen.

Ein besonderes Highlight der Seminarreihe des HPI mit renommierten nationalen und internationalen Sprechern stellt die 2006 ins Leben gerufene Heinrich-Pette-Lecture dar. Im Jahr 2007 wurde die Heinrich-Pette-Lecture von Prof. Dr. Thomas Shenk von der Princeton-University, USA, gehalten. Seine Einladung als Sprecher erfolgte in Anerkennung seiner Verdienste zum Verständnis von Virus-Wirts-Wechselwirkungen.

Die zweite Hamburger „Nacht des Wissens" fand am 9. Juni 2007 statt. Die Beteiligung des HPI an diesem Ereignis war ein voller Erfolg. Vom frühen Abend bis nach Mitternacht suchten fast 900 – zum größten Teil junge – Besucher unser Institut auf und zeigten sich begeistert von den vielfältigen Demonstrationen, Mitmach-Aktionen, Vorträgen und Video-Projektionen zum Thema „Faszination Virusforschung".

Vom 16. bis 19. September 2007 trafen sich ca. 1.000 Hamburger Schüler zum Biotec-Schülerkongress „Faszination Biowissenschaften". Das HPI war über seinen Vorstandsreferenten Dr. Jörg Maxton-Küchenmeister zentral in die Organisation dieses Ereignisses eingebunden und beteiligte sich an den vielfältigen Aktionen sowohl durch Vorträge von HPI-Mitarbeitern als auch durch Präsentation von Laborarbeiten.

Der Schülerkongress war eine Begleitveranstaltung zur diesjährigen Herbsttagung „Molecular Life Sciences 2007" der Gesellschaft für Biochemie und Molekularbiologie, an deren Organisation die Abteilungsleiter des HPI sowie die Leiterin der Forschungsgruppe „Molekulare Pathologie", Dr. Carol Stocking, mitwirkten und – während der Tagung – auch als „Chairpersons" beteiligt waren.

Prof. Dr Wolfgang Deppert vom HPI organisierte zusammen mit Prof. Schäfer (Berlin), Prof. Ishikawa (Kyoto) und Prof. Miyazano (Tokio) den „11.German-Japanese Cancer Workshop“, der vom 29. November bis 1. Dezember 2007 in Kyoto, Japan, stattfand. Auf diesem interdisziplinären Workshop diskutierten führende Wissenschaftler aus Japan und Deutschland aktuelle Fragen der Krebsforschung.

Netzwerke

Das im Jahr 2005 ins Leben gerufene „Leibniz-Zentrum für Infektionsforschung“ (LZIF) ist ein organisatorischer Zusammenschluss des Heinrich-Pette-Instituts, des Bernhard-Nocht-Instituts (BNI) und des Forschungszentrums Borstel (FZB) als Partner-Institute. Der Zusammenschluss hat zum Ziel, gemeinsame Projekte der Partner-Institute zu initiieren und zu fördern sowie das durch den Zusammenschluss erweiterte Methodenspektrum gemeinsam zu nutzen und gezielt Anreize zur institutsübergreifenden Zusammenarbeit zu schaffen. Eine nicht unwichtige Rolle spielt dabei auch die Weiterentwicklung der exzellenten Forschungsergebnisse der Partner-Institute in Richtung wirtschaftlicher Verwertung bzw. medizinischer Anwendung. Dr. Heinrich Hohenberg, wissenschaftlicher Direktor des HPI, wurde bis Ende 2009 als Sprecher des Verbunds wiedergewählt. Für das LZIF wurde jetzt von der Behörde für Wissenschaft und Forschung der Freien und Hansestadt Hamburg eine Koordinierungsstelle bewilligt, die am HPI angesiedelt werden wird.

Program Area „Virus-Host Interaction“

Head: Prof. Hans Will

Introduction

Viruses are obligatory parasites and can produce progeny only after successful infection of permissive host cells. During this process they depend on the availability of many cellular components and functions. Specific and non-specific interactions between viral and cellular factors during the early and late stages of infection determine whether a viral infection will be transient, becomes chronic or eventually leads to non-productive viral latency. The severity and type of virus infection-associated/induced diseases also depend on complex and dynamic interplays of viral components with cell-, organ- and species-specific factors and on the modulation of cellular signalling pathways. Unfortunately, our understanding of the factors and mechanisms involved in virus-host interactions is very limited. This is in part due to distinct differences that have evolved between the large varieties of known viruses on the one hand and the complexity and genetic heterogeneity of the host species on the other hand. However, we are optimistic that our current knowledge in this field can be greatly increased through the use of cellular and animal models in order to reach the short and long term goals of prevention and eradication of infections of pathogenic viruses of global importance as well major improvements in the therapy of currently non-curable disastrous virus infections and associated diseases.

The major aims of our current research program on „Virus-Host Interaction“ are the development of innovative strategies that lead to the elimination or functional inactivation of viruses chronically infecting humans or animals, as well as to the identification of novel targets for effective therapy for virus-associated diseases. In addition, the establishment of novel approaches for the prevention of *de novo* virus infections and re-activation of latent viruses are important goals of our research program. In order to achieve these goals, substantial research activity is directed towards the identification and detailed characterization at the molecular level of both viral and host cell components, which are crucial determinants for the establishment of viral infections and associated diseases. In addition, an essential part of the work program is to decipher the complexity and dynamics of interactions between these cellular and viral components critical at various stages of the life cycle of various viruses and for the development of virus infection-associated diseases, by using the most advanced techniques available.

Focus of our research in 2007 included studies on human immunodeficiency virus type 1 (HIV-I) causing AIDS, related animal viruses causing leukemia and other diseases, human Kaposi-sarcoma associated gamma herpes virus (KSHV), as well as Epstein-Barr virus (EBV) and Cytomegaloviruses (CMV), tumorigenic adeno- and papova-viruses, human and animal hepatitis B viruses (HBV) causing liver diseases and hepatocellular carcinoma.

The major research activities in 2007 in the program area „Virus-Host Interaction“ can be roughly divided into three research fields and have been sorted below accordingly.

I. Determinants of Productive and Latent Virus Infections
II. Use and Modulation of Cellular Functions by Viruses
III. Virus Pathogenicity and Antiviral Therapy

Significant scientific progress was made in 2007 in all three fields of the „Virus-Host Interaction“ research program, as evidenced best from the many excellent publications resulting from our work (see abstracts below and full text of publications via Pubmed).

Many of the publications are frequently cited in research journals with high and very high international reputation. Collaborative work of scientists from our institute and the Max-Planck-Institute of Cell Biology and Genetics in Dresden pubished 2007 in Science lays the technical foundation for a novel therapeutic approach that one day might be used to eradicate the HIV genome from host cells of infected patients. This work has attracted huge and world wide attention in the public media. Additional information on this and most of our other research projects is available on our website and in the annual report 2005/2006, which can be downloaded from our website (www.hpi-hamburg.de).

Note, many projects assigned to the second research program area at the HPI, designated „Cellular Dysregulation", are tightly interconnected both experimentally and intellectually and provide further information on functions of cellular proteins affected by viruses. A large part of the research in both research areas depends on and is funded by external grants from national and international research funding agencies and foundations. The successfully acquisition of these funds is based on our previous and current research achievements.

Research Projects

I. Determinants of Productive and Latent Virus Infections

Efficient generation of adenovirus type 5 vectors by direct cloning into the proviral genome

T. Dobner, P. Groitl

Adenovirus vectors are among the most promising systems for efficient gene transfer into mammalian cells. However, despite recent improvements, the construction of recombinant adenovirus vectors remains a time-consuming procedure which requires extensive manipulations of the viral genome in both *E. coli* and eukaryotic cells. Here we describe a novel strategy that simplifies the generation and production of such viruses. Briefly, a recombinant adenoviral plasmid is generated by direct cloning of expression cassettes derived from all early (E1 – E4) and late (L1 – L5) regions into the viral backbone. After transfection of those plasmids into a mammalian helper cell line, homogenous and high-titer virus stocks are obtained without further plaque purification. The advantages of this technology are multiple: (i) all cloning steps are carried out in *E. coli*, (ii) any genetic region of the viral genome can be specifically modified or deleted, (iii) foreign genes can be introduced into E1, E3 and E4 regions, (iv) expression of therapeutic genes can be controlled by cell-type specific and/or inducible promoters, and, (v) transfection of the linearized viral DNA into permissive cells generates plaques containing only pure virus particles. The efficiency and flexibility of this system is illustrated by the cloning of an wild-type Ad5 bacmid, the insertion of suitable restriction sites into the viral backbone and the successful generation of more than 100 different adenovirus vectors containing defined mutations in viral early and late regulatory proteins, such as E1B-55K, E3-14.7K, E4orf3, E4orf4, E4orf6 and L4-100K.

Supported by ReForMC, University of Regensburg

A 49K isoform of the adenovirus E1B-55K protein is sufficient to support viral replication

T. Dobner, P. Groitl, K. Kindsmüller, S. Schreiner

The adenovirus (Ad) E1B-55K protein is a multifunctional regulator of virus replication that participates in many processes required for maximal virus production, including shut-off of host cell protein synthesis, selective nucleocytoplasmic viral mRNA transport and late protein synthesis as well as and proteolytic degradation of p53 and Mre11, a key component of the cellular DNA double-strand break repair. To further elucidate the role of 55K in lytic infection we generated a virus mutant (H5*pm*4133) carrying stop codons after the second and seventh codon of the 55K reading frame. Unexpectedly, phenotypic studies of H5*pm*4133 in HeLa and A549 cells revealed that it fully exhibits the characteristics of wild-type (wt) Ad in all assays tested. Protein analyses demonstrated that H5*pm*4133 expresses extremely low levels of a ~49K N-terminal truncated product. Significantly, low levels of the 49K isoform were also detected in wt-infected cells, indicating that this E1B protein is generated by reinitiation of translation at in-frame AUGs of the E1B mRNA. These data demonstrate for the first time that the E1B transcription unit encodes a 49K isoform, which is sufficient to proceed through the productive infection in human tumor cell lines.

Steroid dependent control of HIV replication

W. Bohn, R. Reimer, D. Schwarck, K. Wiegers

Infection of resting peripheral mononuclear blood cells (PBMCs) with HIV-1 is not productive due to a block prior to integration of the provirus into the host genome. We found that a unique restriction is determined by the status of the glucocorticoid receptor (GR). Proviral integration increases after addition of a GR ligand. The ligand dependent effect is confined to an early time period after infection and requires GR and the GR binding viral protein Vpr. Ectopic Vpr and GR are localized in the cytoplasm in unstimulated PMCs and comigrate into the nucleus upon ligand addition. Thus, predominant cytoplasmic localization of GR seems to be a specific obstacle for HIV replication. Accordingly, efficient proviral integration in a cell line with a constitutive cytoplasmic GR requires addition of a GR ligand. The data suggest that steroids can overcome the restriction on HIV provirus formation and thereby increase the reservoir of virus producing cells.

Insights into the Selective Activation of Alternatively Used Splice Acceptors by the Human Immunodeficiency Virus Type 1 Bidirectional Splicing Enhancer

C. Asang (1), I. Hauber (2), H. Schaal (1)
(1) Institute for Virology, University Düsseldorf
(2) Heinrich-Pette-Institute, Hamburg

The guanosine-adenosine-rich exonic splicing enhancer (GAR ESE) identified in exon 5 of the human immunodeficiency virus type-1 (HIV-1) pre-mRNA activates either an enhancer-dependent 5'splice site (ss) or 3'ss in 1 intron reporter constructs in the presence of the SR proteins SF2/ASF2 and SRp40. Characterizing the mode of action of the GAR ESE inside the internal HIV-1 exon5 we found that this enhancer fulfils a dual splicing regulatory function (i) by synergistically mediating exon recognition through its individual SR protein binding sites and (ii) by conferring 3'ss selectivity within the 3'ss cluster preceding exon 5. Both functions depend upon the GAR ESE, U1snRNP binding at the downstream 5'ss D4, and the E42 sequence located between these elements. Therefore, a network of cross-exon interactions appears to regulate splicing of the alternative exons4a and 5. As the GAR ESE-mediated activation of the upstream 3'ss cluster also is essential for the processing of intron-containing *vpu/env*-mRNAs during intermediate viral gene expression, the GAR enhancer substantially contributes to the regulation of viral replication.

A Minimal uORF Within the HIV-1 vpu Leader Allows Efficient Translation Initiation at the Downstream env AUG

J.L. Anderson (2), I. Hauber (3), J. Hauber (3), A.T. Johnson (2), S. Kammler (1), J. Krummheuer (1), D.F.J. Purcell (2), H. Schaal (1)
(1) Institute for Virology, University Düsseldorf
(2) Microbiology and Immunology Department, University of Melbourne, Australia
(3) Heinrich-Pette-Institute, Hamburg

Translation of HIV-1 mRNAs generally follows the scanning mechanism. However, by using subgenomic *env* expression vectors, we found translation of glycoprotein from polycistronic

mRNAs were inconsistent with leaky scanning. Close inspection of the leader sequences suggested expression of Env was modulated by an upstream open reading frame (uORF) and a minimal uORF consisting only of a start and stop codon that overlaps with the vpu start site. By altering the strength of upstream AUGs, the length of their coding region and the intercistronic distance, we demonstrate that each of these parameters influenced downstream translation. The minimal uORF is conserved throughout almost all HIV-1 subtypes, despite residing in a non-coding region of the genomic RNA. Mutating the translational start and stop codons of this uORF resulted in up to five-fold reduction in Env expression. Removing the *vpu* uORF and increasing the strength of the authentic *vpu* initiation sequence abolished Env expression from subgenomic constructs and replication of HIV-1, whereas an identical increase in the strength of the minimal uORF initiation site did not alter *env*-expression.

Supported by Deutsche Forschungsgemeinschaft (DFG)

Establishment and Maintenance of Latent Infection by Kaposi's sarcoma-associated herpesvirus

A. Grundhoff, T. Günther, U. Tessmer

During herpesvirus latency, only a small number of viral genes are expressed and the virus persists as an autonomously replicating episome in the nucleus of the infected host cell. Although establishment of latent infection is a critical phase in the viral lifecycle, little is known about the mechanisms which govern this process. We are employing Kaposi's sarcoma-associated herpesvirus (KSHV), a member of the gammaherpesvirus family which is linked to several tumors of endothelial and B cell origin, as a model system to study these processes. While permissive cells (e.g., endothelial cell lines) can be infected with an efficieny of almost 100%, only a small minority of cells becomes stably infected, whereas the bulk of the cells loose the viral episomes over a period of just a few weeks. Thus, contrary to common belief, a latent gene expression profile alone is not sufficient for the establishment of long-term latent infection. Rather, the viral episomes need to be stabilized by events which occur only rarely, or only in rare cells. Analysis of long-term infected single cell clones and artificial KSHV replicons indicate that these events represent epigenetic modifications of the viral episomes themselves. These modifications might be required for efficient episome replication, their segregation during cell division, or both. In addition, they might be required to maintain latent gene expression profiles. Analysis of KSHV virions indicate that the packaged genomes are epigenetically naïve, thereby explaining why such modifications have to be re-established during each infection cycle. The precise nature and location of these modifications remain to be established. We are investigating the DNA methylation and histone modification status of various regions of the KSHV genome at different time points post infection. While our preliminary results suggest that heterochromatin deposition might indeed form the basis for episome loss, a more in-depth study is required to unravel the role of epigenetic marks during KSHV infection. We thus aim to generate a high-resolution temporal and spatial map of epigenetic changes across the entire viral genome. We are also investigating whether specific cellular factors (e.g. PML) alter the epigenetic status of KSHV episomes. Identification of such factors and global pinpointing of epigenetic alterations will shed light on the processes underlying establishment of stable latency.

Avian Hepatitis B Viruses: Molecular and Cellular Virology, Phylogenesis, and Host Tropism

A. Funk, M. Mhamdi, H. Sirma, H. Will

The human hepatitis B virus (HBV) and the duck hepatitis B virus (DHBV) share several fundamental features. Both viruses have a partially double-stranded DNA genome that is replicated via a RNA intermediate and the coding open reading frames (ORFs) overlap extensively. In addition, the genomic and structural organization, as well as replication and biological characteristics, are very similar in both viruses. Most of the key features of hepadnaviral infection were first discovered in the DHBV model system and subsequently confirmed for HBV. There are, however, several differences between human HBV and DHBV. This review will focus on the molecular and cellular biology, evolution, and host adaptation of the avian hepatitis B viruses with particular emphasis on DHBV as a model system.

Supported by Deutsche Forschungsgemeinschaft and Bundesministerium für Bildung und Forschung

Assembly and Budding of a Hepatitis B Virus is mediated by a Novel Type of Intracellular Vesicles.

A. Funk, H. Hohenberg, M. Mhamdi, H. Sirma, H. Will

Formation of enveloped viruses involves assembly and budding at cellular membranes. In this study, we elucidated the morphogenesis of hepadnaviruses on the ultrastructural and biochemical level using duck hepatitis B virus (DHBV) as a model system. Formation of virus progeny initiates at the endoplasmic reticulum (ER) and is conserved both in vitro and in vivo. The morphogenesis proceeds via membrane-surrounded vesicles containing both virions and subviral particles, indicating a common morphogenetic pathway. The virus particle-containing vesicles (VCVs) are generated and maintained by reorganization of endomembranes accompanied by a striking disorganization of the rough ER (rER). VCVs are novel organelles with unique identity and properties of ER, intermediate compartment, endosomes, and multivesicular bodies. VCVs are dynamic structures whose size and shape are regulated by both membrane fusion and fission. Conclusion: Our data indicate a strong reorganization of endomembranes during DHBV infection, resulting in the biogenesis of novel organelles serving as multifunctional platforms for assembly and budding of virus progeny.

Supported by Deutsche Forschungsgemeinschaft (DFG), Stiftung des Deutschen Volkes and Bundesministerium für Bildung und Forschung

Requirement of Activation for Hepatitis B Virus Infection

M. Bruns, C. Maenz

Although in vitro models of human hepatitis B virus replication are established, so far none could approximate infection efficiency as expected from in vivo observations. Susceptibility for HBV infections has only been reported for primary hepatocytes of human, chimpanzee or Tupaia belangeri and the cell line HepaRG. Here we show that the insusceptible human

hepatoma cell line HepG2 can be infected, when the virus was beforehand activated by passage over whole duck liver cell cultures. That suggests an activation step to be performed by specialized liver cells.

Supported by the Deutsche Forschungsgemeinschaft (DFG)

Enzymatic treatment of duck hepatitis B virus: topology of the surface proteins for virions and noninfectious subviral particles

M. Bruns, C. Maenz, U. Matschl

The large surface antigen L of duck hepatitis B virus exhibits a mixed topology with the preS domains of the protein alternatively exposed to the particles' interior or exterior. After separating virions from subviral particles (SVPs), we compared their L topologies and showed that both particle types exhibit the same amount of L with the following differences: first, preS of intact virions was enzymatically digested with chymotrypsin, whereas in SVPs only half of preS was accessible; second, phosphorylation of L at S118 was completely removed by phosphatase treatment only in virions; third, iodine-125 labeling disclosed a higher ratio of exposed preS to S domains in virions compared to SVPs. These data point towards different surface architectures of virions and SVPs. Because the preS domain acts in binding to a cellular receptor of hepatocytes, our findings implicate the exclusion of SVPs as competitors for the receptor binding and entry of virions.

Supported by the Deutsche Forschungsgemeinschaft (DFG)

Entry of Duck Hepatitis B Virus into Primary Duck Liver and Kidney Cells after Uncovery of a Fusogenic Region within the Large Surface Protein

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Hepatitis B viruses exhibit a narrow host range specificity that is believed to be mediated by a domain of the large surface protein, designated L. For duck hepatitis B virus it has been shown that the preS domain of L binds to carboxypeptidase D, a cellular receptor present in many species on a wide variety of cell types. Nonetheless only hepatocytes become infected. It so far remained vague, which viral features procure host range specificity and organotropicity. Using chymotrypsin to treat duck hepatitis B virus we addressed the question, whether a putative fusogenic region within the amino-terminal end of the small surface protein may participate in viral entry and possibly constitute one of the requirements for the host range of the virus. The addition of the enzyme to virions resulted in an increased infectivity. Remarkably, even remnants of enzyme-treated subviral particles proved to be inhibitory for infection. A noninfectious deletion mutant, devoid of the binding region for carboxypeptidase D, could be rendered infectious for primary duck hepatocytes by treatment with chymotrypsin. Although due to the protease treatment mutant and wild-type viruses may have become infectious in an unspecific and receptor-independent manner, the host range specificity was not affected as shown by the inability of viral replication in different hepatoma cell-lines as well as primary chicken hepatocytes. Instead, the organo-

tropicity could be reduced, which was demonstrated by infection of primary duck kidney cells.

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II. Use and Modulation of Cellular Functions by Viruses

Identification and characterization of virally encoded miRNAs

T. Christalla, A. Grundhoff, U. Tessmer, N. Walz

microRNAs (miRNAs), small (~22 nt) non-coding RNAs, are important regulators of cellular gene expression due to their ability to regulate the expression of target mRNA transcripts. Recently, we have developped a combined computational/microarray method for the identification of miRNA-precursors specifically in viral genomes. Using this approach, we were able to identify several miRNAs encoded by the gammaherpesviruses Epstein-Barr Virus (EBV, 25 miRNAs), Kaposi's sarcoma-associated herpesvirus (KSHV, 13 miRNAs) and rhesus Lymphocryptovirus (rLCV, 26 miRNAs). The miRNAs in these viruses are produced from highly spliced transcripts which are expressed during latent infection. Since latency products are thought to play a major role in the development of EBV- and KSHV-associated cancers, we hypothesize that modulation of cellular gene expression by viral miRNAs might contribute to the onset and/or progression of tumorigenesis. To date, the precise targets of EBV- and KSHV-encoded miRNAs remain unknown. We are employing a combination of bioinformatic and experimental approaches to identify such targets. For bioinformatic target prediction, we have developed an algorithm which, in addition to primary sequence information, evaluates the positional and structural context of miRNA target sites, as well as cooperative effects between multiple sites. A genome wide screen for candidate transcripts targeted by EBV- and KSHV-miRNAs produces a list significantly enriched in factors involved in apoptosis and anti-viral host response pathways. Our prediction methods are complemented by unbiased proteomic and genomic screens for proteins/transcripts which are downregulated in cells which express solitary or clustered viral miRNAs. Together with investigation of the phenotypic consequences of ectopic miRNA expression, these experiments aim to clarify the role of KSHV and EBV miRNAs in the viral lifecycle as well as their potential to contribute to the development of herpesvirus-associated malignancies.

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Crossing the Species Barrier: Role of Env–Receptor Interactions in Gammaretrovirus Infections

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The gammaretroviruses compromise a genus of exogenous and endogenous viruses that are widely spread in vertebrates. Analysis of endogenous retrovirus (ERVs) in the genomes of

humans, mice, and other species indicates a longstanding association, probably dating back several hundred million years, during which retroviruses have repeatedly colonized host genomes. Phylogenetic studies of class I (gamma and epsilon) and class II (alpha, beta, delta and lenti) ERVs suggest that horizontal transfer of infectious virus between vertebrate classes occurs only rarely, although several important examples have occurred. In addition to the well-known jumping of a lentivirus from chimpanzees (SIVcpz) to humans (HIV-1), a recent report has demonstrated the first bonafide human infection with a xenotropic MLV-related gammaretrovirus (XMRV) (Urisman et al., 2006). Using retroviral pseudotype assays and receptor binding assays, we have demonstrated that XMRV possesses a wide host range and efficiently infects feral mouse cells as well as established human cell lines and human primary cells. Interference assays confirm the classification of XMRV as a xenotropic MLV-like virus, which use the transmembrane protein Xpr1 as a receptor. Interestingly, XMRV preferentially uses the human versus the murine Xpr1 variant, arguing that XMRV already persists for a longer period in the human population.

A second example is a Koala gammaretrovirus (KoLV), which is currently "invading" the host genome. This virus is closely related to the gibbon ape leukemia virus (GALV), although the vector responsible for transmission of the virus between these two species with distinct habitats has not been conclusively identified. We have recently isolated an ERV from *Mus cervicolor,* a mouse strain found in Southeast Asia that is closely related to the GALV and KoLV isolates and thus may represent the common ancestor. Host spectrum analysis show that this virus has lost its ability to infect murine cells but can efficiently infect other vertebrate cells (e.g. human, ape, and mink), suggesting that a change in Env sequences has permitted its interspecies transmission.

Identification of functional domains in the adenovirus E1B-55K protein required for binding to and degradation of cellular Mre11

T. Dobner, K. Kindsmüller, M. Schmid

The human subgroup C adenoviral E1B-55K protein is a multifunctional regulator of cell cycle-independent adenovirus (Ad) replication that controls several processes required for maximal virus production, including selective viral late mRNA transport to the cytoplasm, shut-off of host cell protein synthesis, and, proteolytic degradation of p53 and Mre11, a key component of the mammalian DNA double-strand break repair Mre11/Rad50/Nbs1 (MRN) complex. Accumulating evidence suggests that the latter activity relies on direct interactions of E1B-55K with both cellular factors, and the ability of E4orf6 to contact components of an E3 ubiquitin ligase complex.

In order to elucidate the role of 55K in E4orf6/E1B-55K-mediated degradation of Mre11 we have generated a panel of Ad mutants carrying defined amino acid exchanges in different regions of the viral protein, including the CRM1-dependent nuclear export signal (NES). These were tested for their ability to interact with and to target Mre11 for proteolytic degradation in combination with E4orf6. Our studies show that amino acid substitutions in the amino-terminal region encompassing the NES and in the central part have no or only moderate effects on Mre11-binding and -degradation. By contrast, two amino acids exchanges in the C-terminal region completely abolish the interaction with Mre11 and efficient degradation of the cellular protein during the course of the infection. Interestingly, the inability of

this mutant protein to destabilize Mre11 also correlated with a dramatic reduction of E4orf6 steady-state levels. These results indicate that 55K regulates the stability of E4orf6 in infected cells and suggest that this activity is dependent on its interaction with Mre11 and/or the MRN complex.

Control of mRNA export by adenovirus E4orf6 and E1B-55K proteins during productive infection requires the E4orf6 ubiquitin ligase activity

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During the adenovirus infectious cycle, the early proteins E4orf6 and E1B-55K are known to perform several functions. These include the nuclear export of the late viral mRNAs, the block of nuclear export of the bulk of cellular mRNAs, and the ubiquitin-mediated degradation of selected proteins, including p53 and Mre11. While investigating the mechanism of the degradation function, it was discovered that the E4orf6 product assembles with cellular proteins to form an E3 ubiquitin ligase complex. Through the presence of BC-boxes, E4orf6 is able to compete with cellular proteins for binding to Elongins B and C in order to assemble a Cul5-based ligase complex. E1B-55K, which has been known for some time to associate with the E4orf6 protein, is thought to bind to specific substrate proteins to bring them to the complex for ubiquitination. Earlier studies with E4orf6 mutants indicated that the interaction between the E4orf6 and E1B-55K proteins is optimal only when E4orf6 is able to form the ligase complex. These and other observations suggest that most if not all of the functions ascribed to E4orf6 and E1B-55K during infection, including the control of mRNA export, are achieved through the degradation of specific substrates by the E4orf6 ubiquitin ligase activity. We have tested this hypothesis through the generation of a virus mutant in which the E4orf6 product is unable to form a ligase complex and indeed have found that this mutant is incapable both of promoting late viral mRNA export and of blocking the export of cellular mRNAs.

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Intranuclear targeting and nuclear export of the adenovirus E1B-55K protein is regulated by SUMO1 conjugation

T. Dobner, P. Groitl, B. Härtl, J. Hauber, K. Kindsmüller

In this study we have investigated the requirements for CRM1-mediated nuclear export and SUMO1 conjugation of the adenovirus E1B-55K protein during productive infection. Our data show that CRM1 is the major export receptor for E1B-55K in infected cells. Functional inactivation of the E1B-55K CRM1-dependent nuclear export signal (NES) or leptomycin B treatment causes an almost complete redistribution of the viral protein from the cytoplasm to the nucleus, and its accumulation at the periphery of the viral replication centers. Interestingly, however, this nuclear restriction imposed upon the wild-type and the NES mutant protein is fully compensated by concurrent inactivation of the adjacent SUMO1 conjugation site. Moreover, the same mutation fully reverses defects of the NES mutant in the

nucleocytoplasmic transport of Mre11 and proteasomal degradation of p53. These results show that nuclear export of E1B-55K in infected cells occurs via CRM1-dependent and -independent pathways, and suggest that SUMO1 conjugation and deconjugation provides a molecular switch that commits E1B-55K to a CRM1-independent export pathway.

Analysis of human adenovirus E1B-55K and E4orf6 nucleocytoplasmic shuttling functions in productive infection

T. Dobner, K. Kindsmüller

The adenovirus type 5 (Ad5) E1B-55K and E4orf6 proteins are multifunctional regulators of Ad5 replication participating in many processes required for maximal virus production. A complex containing the two proteins has been implicated in the proteolytic degradation of the tumor suppressor protein p53, shut-off of host cell protein synthesis and selective viral late mRNA transport from the nucleus to the cytoplasm. It has been proposed that the latter activity may involve a CRM1-dependent nuclear export pathway since both proteins continuously shuttle between the nuclear and cytoplasmic compartments via leucine-rich nuclear export signals (NESs), and because mutations in the E4orf6 NES negatively affect late viral gene expression in artificial transfection/infection complementation assays. In contrast a different conclusion was drawn from similar studies showing that E1B-55K/E4orf6 promote late gene expression without intact NESs or active CRM1. To evaluate the role of CRM1-dependent nuclear export of E1B-55K/E4orf6 for efficient late viral gene expression and virus production in the normal context of Ad-infected cells we generated virus mutants carrying amino acid exchanges in the NESs of E1B-55K, E4orf6 and both proteins. Pheno¬typic analyses in A549 and HeLa cells revealed that mutations in the NESs of E1B-55K or E4orf6 had no or only moderate effects on viral DNA synthesis, late viral protein expression and virus growth. Similar results were observed in cells infected with the double NES mutant virus. These results demonstrate that CRM1-mediated nuclear export of E1B-55K, E4orf6 and/or the E1B/E4orf6 protein complex is not essential for expression of viral late genes.

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Inhibition of TNF receptor 1 internalization by adenovirus 14.7K as a novel immune escape mechanism

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The adenoviral protein E3-14.7K (14.7K) is an inhibitor of TNF-induced apoptosis, but the molecular mechanism underlying this protective effect has not yet been explained exhaustively. TNF-mediated apoptosis is initiated by ligand-induced recruitment of TNF receptor–associated death domain (TRADD), Fas-associated death domain (FADD), and caspase-8 to the death domain of TNF receptor 1 (TNFR1), thereby establishing the death-inducing signaling complex (DISC). Here we report that adenovirus 14.7K protein inhibits ligand-induced TNFR1 internalization. Analysis of purified magnetically labeled TNFR1 complexes from murine and human cells stably transduced with 14.7K revealed that prevention of TNFR1 internalization resulted in inhibition of DISC formation. In contrast, 14.7K did not affect TNF-

induced NF-κB activation via recruitment of receptor-interacting protein 1 (RIP-1) and TNF receptor–associated factor 2 (TRAF-2). Inhibition of endocytosis by 14.7K was effected by failure of coordinated temporal and spatial assembly of essential components of the endocytic machinery such as Rab5 and dynamin 2 at the site of the activated TNFR1. Furthermore, we found that the same TNF defense mechanisms were instrumental in protecting wild-type adenovirus-infected human cells expressing 14.7K. In sum these studies will reveal new molecular mechanisms implemented by a virus to escape immunosurveillance by selectively targeting TNFR1 endocytosis to prevent TNF-induced DISC formation.

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High resolution electron microscopical (EM)-analysis of the distribution of TNF receptor 1 and the inhibition its internalization using immunogold stereo surface replica techniques

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The adenoviral protein E3-14.7K is an inhibitor of TNF-induced apoptosis, but the molecular and structural mechanism underlying this protective effect has not been explained so far. TNF-mediated apoptosis is initiated by ligand-induced recruitment of TNF receptor–associated death domain (TRADD), Fas-associated death domain (FADD), and caspase-8 to the death domain of TNF receptor 1 (TNFR1), thereby establishing the death-inducing signaling complex (DISC). In the first step of our EM-study we analyse the exact distribution of TNF receptors at the plasma membrane surface of adenovirus–infected human cells applying the heavy metal immunogold surface replica technique. In a second step we will investigate by 3D ultrathin section reconstruction techniques the more complex intracytoplasmatical mechanisms that allow the adenovirus 14.7K protein to inhibit ligand-induced TNFR1 internalization.

Arginine methylation of human adenovirus type 5 L4-100K protein is required for efficient virus replication

O. Dirlik-Koyuncu, T. Dobner

The adenovirus type 5 (Ad5) L4-100K nonstructural protein (100K) is required for inhibition of cellular protein synthesis and selective translation of tripartite leader containing viral late mRNAs by ribosome shunting. In addition, 100K has been implicated in the trimerization and nuclear localization of hexon monomers. We have previously shown that 100K is a substrate of the protein arginine methylation machinery, an emergent post-translational modification system involved in a growing number of cellular processes, including transcriptional regulation, cell signaling, RNA processing and DNA repair. As understood at present 100K arginine methylation involves protein arginine methyltransferase 1 (PRMT1), which asymmetrically dimethylates arginines embedded in an arginine-glycine-glycine (RGG) motif. To assess the role of 100K arginine methylation on its lytic functions we examined the effect of amino acid substitutions in the RGG motif in the context of Ad-infected cells by

generating and testing the appropriate virus mutant. Results from these studies revealed that the mutations substantially reduce late viral protein expression and virus growth demonstrating that 100K protein arginine methylation is an important host-cell function required for efficient Ad replication. Biochemical and immunofluorescence analyses indicate that PRMT1-catalyzed methylation of arginine residues in the RGG motif regulates the binding of 100K to hexon in the cytoplasm and promotes the nuclear localization of the structural protein. These data confirm the role of 100K as a transport and scaffold protein for hexon and indicate that this activity is regulated by arginine methylation.

Ultrastructural analysis of cytomegalovirus tegument protein interactions

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Cytomegalovirus tegument proteins are critical constituents of the virus particle that play a role in particle assembly, virus entry and exit, establishment of infection and immunomodulation. Based on an established map of protein interactions between tegument proteins, which provides a scaffold for further definition of virus structure assembly and function, the main focus of the investigations is the interaction between ppUL82 (pp71) and ppUL35. Both proteins localize to PML oncogenic domains and cooperatively transactivate the immediate-early enhancer of HCMV. In addition, deletion of the UL35 gene affects virus assembly by modulating nucleocytoplasmic localization of other tegument proteins. Thus, ppUL35 is part of a cell-biological switch critical for immediate-early regulation and virus assembly. The use of Carbocell microreactors for sample preparation and ultrastructural analysis assures the perpetuation of virus components *in situ* during the whole investigation process. Cryofixation by high pressure freezing and deep temperature substitution are used to preserve the tegument proteins in their native state for further investigation by immunogold-labelling and high resolution transmission electron microscopy.

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Elucidation of the SV40-induced DNA damage signaling cascades and their components utilized by SV40

I. Dorneiter, K. Korf, A. Mena-Nunez, G. Rohaly, H. Will

We and others could show that SV40 infection of CV-1 cells activates the ATM-mediated signaling pathway leading to ATM-catalyzed phosphorylation of T-Ag on S120, an event required to support viral DNA replication. Activation of ATM occurs by autophosphorylation and depends on the Mre11-Rad50-Nbs1 (MRN) complex, which recruits ATM to sites of DNA damage via interaction between ATM and the C-terminus of Nbs1. Additionally, it was reported that T-Ag targets and inactivates the essential component of the ATM-activating MRN complex Nbs1, an event that was found to be essential for maximal viral amplification and viral induced cellular endoreplication. However, the findings are not conclusive and raise the question about the mechanisms that allow ATM activation in the absence of functional MRN. A recent report demonstrated that under certain circumstances ATM could be

activated in the absence of functional MRN through direct ATR-catalyzed phosphorylation. Thus, it is possible that SV40 infection utilizes the same approach to activate the ATM-signaling pathway in the absence of functional MRN. The assumption is supported by our observation that Chk1, a direct substrate of ATR, is already phosphorylated 16 h post infection (hpi) and hence substantially precedes Chk2 phosphorylation at 48 hpi. Data indicate that ATM activation could be preceded by activated ATR. However, the fact that activation of ATR depends on replicative stress, which might be induced by the onset of viral and/or cellular replication, and SV40 replication requires ATM-phosphorylated T-Ag does not support this assumption. Thus, it remains to be elucidated whether the PIK kinases are activated concomitantly or sequentially in a dependent/independent manner and what PIK kinase is responsible for phosphorylating and activating the down-stream effectors Chk1, Chk2, p53, Δp53 and the double strand break marker H2AX. Thus, our goal is to identify the DNA damage response signaling pathways and their components that are purposefully activated and inactivated in SV40-infected permissive cells, to enable adaptation of the host cell's milieu for optimal viral progeny.

Elucidation of the SV40-induced alterations of the host cell's chromatin licensing and replication machinery

I. Dorneiter, K. Korf, A. Mena-Nunez, G. Rohaly, H. Will

Our investigations revealed that SV40 activates the ATM as well as the ATR-mediated DNA damage response pathways to maintain the cell in a replication competent environment and to prevent collapse of replication forks, a situation most favorable for optimal SV40 replication. Indeed, our results demonstrate that SV40 infection leads to S-phase attenuation or arrest, which is accomplished by Δp53-mediated inhibition of S-phase specific cyclin A-Cdk2 activity. However, we could demonstrate that in UV-irradiated cells Δp53-mediated inhibition of cyclin A-Cdk2 activity does not just attenuate S-phase progression it also inhibits DNA replication to allow repair of damaged DNA prior to the onset of replication. In contrast, SV40-infected permissive wtp53 CV-1 cells replicate in the absence of cyclin A-Cdk2 activity and even acquire a >G2 DNA content. Therefore, two questions arise, first of all how does the virus override the inhibitory effect the intra-S-phase checkpoint comprises on DNA replication without jeopardizing S-phase attenuation or arrest and secondly, how does the virus prevail the host's DNA replication licensing and initiation frequency that is usually restricted to once and only once per cell cycle.

We suggest that SV40 overrides DNA replication control through Cdk/cyclin-deregulation, most likely facilitated by activating the ATM/ATR-mediated DNA damage response pathways, which in turn activate cell cycle checkpoints. Besides driving the cell cycle, Cdks and cyclins are also required to promote licensing as well as restricting DNA replication origin firing to once per cell cycle by preventing the assembly of pre-replicative complexes outside of G1-phase. In normal cycling cells cyclin E, an activator of Cdk2, is expressed at the G1/S boundary and promotes events associated with genome replication. Expression of cyclin E is tightly regulated so that peak levels occur at the G1/S boundary followed by a decline and loss of expression during S-phase. However, under certain circumstances cell cycle-dependent turnover of cyclin E is impaired, leading to both accumulation of cyclin E as well as deregulation of expression relative to the cell cycle. Indeed, in SV40-infected cells cyclin E

degradation is prevented, leading to stabilization of cyclin E and accordingly an increase in active cyclin E-Cdk2 complexes that are quite capable to drive viral and cellular DNA replication. In contrast to cyclin E, cyclin A-Cdk2 phosphorylates and therefore inactivates the essential licensing factors Cdc6, Cdt1 and the DNA replication initiation factor Pol α. We could demonstrate that cyclin A-Cdk2 phosphorylation of Pol α abrogates complex formation with the cellular origin binding protein MCM2 as well as the viral origin binding protein SV40 T-Ag, preventing re-loading of the replicase onto the appropriate origin and thus initiation of DNA replication. Together, a model for SV40-induced endoreplication can be depicted whereby stabilization of cyclin E and Δp53-mediated inactivation of cyclin A-Cdk2 would facilitate re-licensing, re-initiation and accordingly endoreplication in arrested S-phase cells. Thus, our goal is to prove that the SV40-induced alteration of the host cell's chromatin licensing and replication machinery is accomplished by utilizing the ATM/ATR-mediated DNA damage response pathways for Cdk deregulation.

III. Virus Pathogenicity and Therapy

HIV-1 Proviral DNA Excision Using an Evolved Recombinase

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HIV-1 integrates into the host chromosome and persists as a provirus flanked by long terminal repeats (LTRs). To date, treatment regimens to combat HIV are based primarily on virus enzyme inhibitors and molecules that inhibit virus-cell fusion. However, none of the current strategies directly target the integrated provirus and hence a cure for HIV infection does not yet exist. To eliminate the integrated provirus, we used substrate linked protein evolution to generate a tailored recombinase, which recognizes an asymmetric sequence within an HIV-l LTR. This evolved recombinase efficiently excises proviral DNA from the genome of infected cells to combat and possibly cure retrovirus-induced diseases through DNA surgery.

In Search for Novel Agents in Therapy of Tropical Diseases and Human Immunodeficiency Virus

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Malaria, sleeping sickness, Chagas' disease, Aleppo boil and AIDS are among the tropical diseases causing millions of infections and cases of deaths per year because only inefficient chemotherapy is available. Since the targeting of the enzymes of the polyamine pathway may provide novel therapy options, it was aimed to inhibit the deoxyhypusine hydroxylase, which is an important step in the biosynthesis of the eukaryotic initiation factor 5A. In order

to identify new lead compounds a library of piperidines was produced and biologically evaluated. 3,5-diethyl piperidone-3,5-dicarboxylates substituted with 4-nitrophenyl rings in 2- and 6-position were found to be active against *Trypanosoma brucei brucei* and *Plasmodium falciparum* combined with low cytotoxicity against macrophages. The corresponding monocarboxylates are only highly active against the *T. brucei brucei*. A piperidine oximether demonstrated the highest plasmodicidal activity. Moreover, selected compounds were also able to inhibit replication of HIV-1.

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Strong and Selective Inhibitors of HBV-Replication among Novel-N4-Hydroxy-and 5-Methyl-beta-L-Deoxycytidine Analogues

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Novel N(4)-hydroxy- and 5-methyl-modified beta-L-deoxycytidine analogues were synthesized and evaluated as anti-hepatitis B virus (HBV) agents. Their in vitro efficiency was investigated in stably HBV-transfected HepG2.2.15 cells. beta-L-2',3'-Didehydro-2',3'-dideoxy-N(4)-hydroxycytidine (beta-L-Hyd4C) was most effective in reducing secreted HBV DNA (EC50=0.03 microM) followed by beta-L-2',3'-dideoxy-3'-thia-N(4)-hydroxycytidine (EC50=0.51 microM), beta-L-2',3'-dideoxy-N(4)-hydroxycytidine (EC50=0.55 microM), and beta-L-5-methyl-2'-deoxycytidine (EC50=0.9 microM). The inhibition of the presumed target, the HBV DNA polymerase, by the triphosphates of some of the beta-L-cytidine derivatives was also assessed. In accordance with the cell culture data, beta-L-Hyd4C triphosphate was the most active inhibitor with an IC50 value of 0.21 microM. The cytotoxicity of some of the 4-NHOH-modified beta-L-nucleosides was dramatically reduced in comparison to the corresponding cytidine analogues with the unmodified 4-NH2-group. The CC50-values for beta-L-Hyd4C in HepG2 and HL-60 cells were 2,500 microM and 3,500 microM, respectively. In summary, our results demonstrate that at least beta-L-Hyd4C recommends as highly efficient and extremely selective inhibitor of HBV replication for further investigations.

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Cooperation of wild-type and mutant p53 with viral oncogenes in tumorigenesis

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The growth arrest and pro-apoptotic functions of wild-type p53 provide a barrier to viral infection and transformation. Papovaviruses and adenoviruses resolve this problem by binding and inactivating p53, accompanied by p53 destruction via proteasomal degradation. SV40 T-antigen (T-Ag) is an exception insofar, as binding of T-Ag to p53 leads to p53 stabilization, indicating that p53 provides some function(s) to T-Ag, which are important for T-Ag mediated cellular transformation. Analysis of T-Ag induced transformation in p53 wild-type expressing BALB/c 3T3 cells and p53-null BALB/c 10-1 cells revealed that transformation is

impaired in the p53-null cells. Further analyses showed that the N-terminal 40 amino acids of p53 harbour the p53 "helper function" for SV40 transformation. The p53 N-terminus is an important platform for the binding of different cellular factors. Of particular interest are the acetylases CBP/p300, as it has been described that T-Ag becomes acetylated by them. Indeed, we found that T-Ag is acetylated in 3T3, but not in 10-1 cells and in 10-1 cells reconstituted with an N-terminally truncated p53. We pursue the hypothesis that the helper function of p53 in SV40 transformation is to target CBP/p300 to T-Ag. The ternary complex T-Ag-p53-CBP might be important in modulating T-Ag mediated cellular transcription to the needs of transformation.

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Analysis of the metastatic cascade using mouse models for mammary adenocarcinoma

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BALB/c WAP-T mice transgenic for the SV40 early gene region under the control of the murine *whey acidic protein (WAP)* promoter represent a suitable model for analysing mammary carcinogenesis. Upon induction by lactotrophic hormones, the transgene is expressed specifically in mammary epithelial cells, and the mice develop intraepithelial neoplasias, which further progress to invasive, but rarely metastatic mammary carcinoma. Intraepithelial neoplasias developed by WAP-T mice closely resemble human DCIS, thereby validating these mice as a suitable model system. By crossing WAP-T (T1) mice with WAP mutp53 mice (BALB/c WAP-mutp53 (H22) mice transgenic for a mutant $p53^{R270H}$ minigene carrying a point mutation equivalent to a human tumor derived hot spot mutation (human $mutp53^{R273H}$ ▯ murine $mutp53^{R270H}$), we assessed the putative oncogenic properties of mutant p53 (mutp53). In order to assess the severity of the disease, we introduced a histological staging and grading system based on the guidelines for murine mammary carcinoma classification edited by the Annapolis consensus. Compared to mono-transgenic T1 mice, bi-transgenic T1-H22 mice showed a higher tumor incidence, bi-transgenic tumors a worse clinical staging, histological grading, and enhanced metastasis, as metastases were found in only 11% of mono-transgenic T1, but in 42% of bi-transgenic T1-H22 mice. Furthermore, the few metastases found on mono-transgenic mice all were of stage M1 (micrometastases, approx. 100 cells per lung section, only detectable under the microscope), whereas bi-transgenic animals also showed metastases of stage M2 diameter < 2 mm), and of stage M3 (diameter > 3 mm). Pulmonary metastasis thus is significantly (Fisher's exact test: P = 0.0312) more frequent in bi-transgenic compared to mono-transgenic mice. The increased metastasis in bi-transgenic mice is the result of an increased invasiveness of the bi-transgenic tumors. While most tumors in T1 mice were not invasive, bi-transgenic tumors metastasize to the lungs, invade the venous blood system, regional lymph nodes, and infiltrate the surrounding musculature.

Analyses of tumor uniformly graded tumors samples for genetic alterations by array CGH and for differences in gene expression by Affymetrix microarray analysis showed a marked difference between low grade (grades 1 and 2) and high grade (grades 3 and 4) tumors. A major genetic alteration differentiating higher grade from lower grade tumors can be found in an area encompassing the *Met* locus. The proportion of high grade tumors with amplification of *Met* and neighboring genes is significantly higher in G3-G4 (15 out 16) than G1-G2 (3 out 16) tumors (Fisher's exact test: $P < 0.001$). The massive amplification of the *Met*-locus could also be visualized by FISH, and it functionality was supported by a strong (50 to 100-fold) overexpression of the *c-Met* mRNA, and an accumulation of the Met protein in tumors. As overexpression of the *c-Met* gene correlates with enhanced invasiveness and metastasis also in human tumors, these data support the suitability of our mouse model to decipher molecular alterations relevant for human tumorigenesis.

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Induction of cellular immune response against SV40 induced mammary carcinoma in transgenic mice

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BALB/c *WAP-T* mice, developing ductal mammary carcinoma, represent a suitable experimental system to study parameters important for induction of cellular immune response. In BALB/c mice, induction of cellular immunity against transplantable murine SV40 tumors can be achieved by pre-immunization with SV40, although BALB/c mice are considered as "low responders" in terms of a specific CTL response towards SV40 T-Ag. However, pre-immunization with live SV40 or SV40 T-Ag did not protect BALB/c *WAP-T* transgenic mice from endogenous mammary carcinogenesis, indicating that these mice acquired tolerance against SV40 T-Ag during embryogenesis. Therefore, we inserted a 33bp oligomer encoding the MHC class I ($H\text{-}2^d$) restricted T-cell epitope of the nucleoprotein of LCM virus, a dominant epitope in BALB/c mice, into the C-terminal coding region of the SV40 T-Ag (*WAP-T-NP* transgenic lines). Expression of this hybrid gene allows analyzing the immune responses after LCM virus infection of *WAP-T-NP* mice expressing the dominant BALB/c NP-epitope of LCM virus. While the *WAP-T-NP* lines are tolerant against SV40, they are not tolerant against LCMV, as these mice mount a cellular immune response against the LCMV-NP epitope. Adoptive transfer of immune competent cells from LCMV-infected BALB/c wild-type mice into *WAP-T-NP* mice strongly reduced the number of T-Ag positive cells in lactating mammary glands. In addition, direct infection of *WAP-T-NP* mice with LCMV resulted in a similarly strong reduction of T-Ag expressing cells. The NP-specificity of the immune reaction could be verified by infection with recombinant vaccinia viruses, coding for the LCMV-NP. In contrast, a vaccinia recombinant encoding the glycoprotein-precursor of LCMV failed. Furthermore, local injection of LCMV into tumors of *WAP-T-NP* mice resulted in an accelerated delayed-type hypersensitivity (DTH) reaction, which presented itself as a significant swelling of the tumor due to infiltrating CTLs, NK cells, and local overproduction of cytokines. The DTH reaction started already within 1 day of infection, followed by a strong reduction of

T-Ag expressing cells. The data strongly suggest that in WAP-T-NP mice the outgrowth of T-Ag expressing cells is controlled, at least in part, by an immune surveillance via NP-epitope specific $CD8^+$ T cells.

Supported by EU FP6

Program Area „Cellular Dysregulation“

Head: Prof. Wolfgang Deppert

Introduction

The multitude and complexity of the regulatory networks operating within and in-between cells renders a cell vulnerable to endogenous and exogenous insults. Endogenous and exogenous cellular stress conditions will threaten the fragile functional balance of a cell, as they can lead to cellular damage at the level of individual organelles, and at level of the genome. It therefore is of vital importance that cells have developed a variety of protective pathways that cope with endogenous or exogenous stress situations, leading either to repair of the damage incurred, or to elimination of the damaged cell by apoptosis. However, despite the elaborate protective arsenals of a cell, there always will be conditions that lead to either transient or permanent non-physiological alterations in cellular signaling characteristic for pathologic alterations. Important examples are viral infections and tumor development. In both conditions cellular safeguards have failed, leading to the re-programming of cellular pathways for the needs of viral replication or for successful tumor evolution. To understand these and other pathologic conditions, it is necessary to study in detail the basic mechanisms of cellular signaling, as well as the safeguard pathways insuring their proper functioning. Often the study of a dysregulated pathway will lead to the understanding of the regular pathway, and vice versa.

Clearly, “cellular dysregulation“ is a very broad topic. However, it is becoming more and more evident that many pathologic conditions lead to alterations in similar major cellular pathways, or violate similar protective actions of the cell. In analyzing these pathways at the cellular level, the distinction between pathways dysregulated by e.g. viral infection or tumor evolution no longer seems reasonable. On the contrary, attacking the problems of cellular dysregulation from different angles will clearly have synergistic effects. Thus the seemingly broad spectrum of projects addressing this topic serves the major goal of understanding the regulatory mechanisms that govern the appropriate functioning of a cell, with the hope that understanding such mechanisms, and how they might become dysregulated under various pathologic conditions, will help us to find handles for either preventing dysregulation or for curing the effects of cellular dysregulation at the level of the organism.

A major research topic within this program area is devoted to understanding the functions and the regulation of the tumor suppressor p53, reflecting our efforts to decipher the role of p53 as *the* central player in coordinating and executing cellular defense and protection mechanisms against endogenous and exogenous cellular stress situations. The finding reported here that the DNA-intercalating drug chloroquine – well known as an anti-malaria drug – can activate the transcriptional response of p53 and induce apoptosis in tumor cells in which this cell death response normally cannot be induced by radio- or chemotherapy underscores the benefit of this approach. P53 also is required to induce cellular senescence, which only recently has been identified as a first-line cellular defense mechanism, stopping the growth of hyperplasia. Interestingly, in contrast to our previous understanding, the tumor suppressor pRb2, and not its siblings pRb and p106, is the major effector protein executing the senescent state. The finding is part of the Ph.D. thesis of Heike Helmbold, who was awarded the Ph.D. graduation prize of the Faculty of Chemistry, University of Hamburg.

Novel mouse models that may help to understand the oncogenic functions of mutant p53 in mammary carcinogenesis, the role of telomerase re-expression in tumors, or why relapsed leukemic malignancies very often are resistant to further treatment despite the tumor cells expressing a functional wild-type p53 have been established. All models hold the promise of yielding results that could be applied in tumor diagnosis and treatment.

The program area “Cellular Dysregulation“ has substantially gained by new projects summarized in section *“III.3 Viral oncogenes”*, page 42 ff. It not only contains projects deciphering the interaction of adenoviral proteins with cellular key regulators, but also a very innovative project on viral microRNAs that are able to exert oncogenic functions.

Posttranslational modifications are key modulators of protein function. In a paper published in the *Proceedings of the National Academy of Science, USA*, a collaborative work between the groups of Prof. Hauber and Prof. Dobner demonstrated that SUMOylation regulates CRM1-independent nuclear export. The exceptional importance of this finding was highlighted by a comment in *Cell*.

Member of the program area “Cellular Dysregulation“ have co-organized the following international meetings: The “Get Univation”, a German-Turkish Meeting to further the cooperation in biotechnology between scientists of the two countries in Braunschweig, February 26, 2007 (Cagatay Günes); “Eleventh Japanese-German Workshop on Molecular and Cellular Aspects of Carcinogenesis”, November 29 – December 1, 2007, Kyoto, Japan: Wolfgang Deppert (co-organized with Reinhold Schäfer, Berlin, Kohei Miyazono, Tokyo, and Fuyuki Ishikawa, Kyoto).

The following short reports document the high standard of research in this program area and the significant progress made during the last year in the individual projects (for further details see Research Report 2005/2006). The large number of publications in scientific journals with a high impact factor best supports this statement.

Research Projects

I. Analysis of p53 Pathways

Chromatin structure and regulation of p53 function

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DNA binding is central to the ability of p53 to function as a tumor suppressor. An important feature that is common to all modes of p53:DNA interactions is the extraordinary sensitivity of p53 to the topology of its target DNA. p53 can activatetranscription of genes with diverse and even antagonistic functions in the regulation of cell cycle, DNA repair or apoptosis. The search for new compounds activating p53 transcriptional responses is an intensively developing field in the area of clinical cancer research. While effectively inducing transient arrest of cell growth in gliomas, many of the known p53 inducers are less efficient in inducing an apoptotic response. The identification of agents that are effective in activating the apoptotic shoulder of the p53 transcriptional response thus is a matter of crucial importance. We have identified chloroquine (ClQ) as a potent activator of p53 transcriptional activity in different types of tumor cells. We show that ClQ inhibits tumor growth and induces a cell death response in gliomas in vitro and in vivo. Furthermore, ClQ sensitizes glioma cells to other cytotoxic treatments currently in clinical use for the treatment of malignant gliomas.

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Transcriptional control of gene expression by wild-type and mutant p53

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Wild-type (wt) p53 is a transcription factor that activates transcription after binding to p53-specific response elements of its target genes.Despite lacking the ability for sequence-specific DNA binding, mutant p53 (mutp53) proteins have been shown to regulate a large number of tumor-associated genes. Although mutp53 no longer displays sequence-specific DNA-binding it has retained an imprived ability to interact with DNA in a strictly DNA-structure-dependent manner. To explore the molecular mechanism of mutp53-specific transcriptional regulation, we used as a model the human glioblastoma cell line U251 expressing mutp53^{R273H}. In glioblastoma, codon 273 is the most frequently mutated codon in the *TP53* gene. Using a whole-genome ChIP approach, we established a library of genomic sequences bound by mutp53 and analyzed 200 of them. Nucleotide analysis revealed that mutp53 binding sites (mutp53BS) display characteristics of MARs, are enriched in repetitive and mobile DNA sequences, and derived from regulatory intronic and intergenic regions, with a preferential accumulation of mutp53BS in the first intron. The most striking observation is a strong enrichment of Alu-type SINE (short interspersed nucleotide element) in the intronic mutp53BS. These repetitive sequences represent ~13% of human genome, are transcriptionally active and fulfil multiple functions in the regulation of gene expression. To asses the biological relevance of mutp53 binding to intronic and intergenic sequences, we generated stable cell clones of U251 cells transfected with shRNA/p53 expression constructs. Expression of genes containing intronic mutp53BS was analyzed by semi-quantitative, quantitative real time PCR and oligonucleotide-based microarray hybridization in parental cells and upon stable shRNA mediated knock-down of mutp53 expression, and correlated with the absence or presence of mutp53. The biological significance of mutp53 expression was confirmed by agar colony growing assay, demonstrating the strongly reduced capacity of cells lacking mutp53 to grow under these conditions. Reduction of the tumorigenic phenotype in mutp53 knock-down cells was also accompanied by a reduced growth rate, and by alterations in the differentiation status of the cells.

We are currently testing the hypothesis that wtp53 also has the capacity to modulate gene expression by interacting with genomic DNA in a strictly structure-dependent fashion. This could explain the extremely large number of wtp53 regulated genes.

Supported by Deutsche Krebshilfe, Deutsche Forschungsgemeinschaft (DFG), EU FP6

Crosstalk between p53 and retinoblastoma family proteins in cellular senescence

W. Bohn, H. Helmbold, N. Kömm

Cellular senescence is activated in response to a variety of stress signals and stalls cells irreversibly in G1/S transition. Appearance of senescent markers *in vivo* in premalignant lesions but not in malignant tumors emphasizes its role as a tumor suppressor mechanism. Cooperative functions of the p53-p21 and p16INK4a-pRb were thought to be of fundamental importance for the induction of cellular senescence. But as we showed recently, wild-type p53 can activate cellular senescence also in rat cells lacking p16INK4a. The cells are arrested precisely in between cyclin E to cyclin A-mediated steps in G1/S transition and accumulate the retinoblastoma protein Rb2 instead of pRb. It suggests that Rb2 is a critical mediator of the senescence response, whereas p16INK4a controlled pathways involving pRb not necessarily must be functional. We now found that also human primary cells and human tumor cell lines required a p53-Rb2 interaction for induction of a senescence growth arrest. It shows that cooperation between Rb2 and p53 is of fundamental importance for activating senescence in human cells, which may also influence cancer therapy outcome.

Supported by Deutsche Krebshilfe

Loss of p53 dependent growth control in glioblastomas

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Malignant gliomas are highly resistant to therapeutic treatment. The common strategy of glioblastoma treatment primarily consists of tumor excision, followed by γ-irradiation and chemotherapy with alkylating agents. But the rate of recurrences is still high and over 90% of the patients die within 2 years after tumor resection.

Most of the glioblastomas are classified as 'primary *(de novo)* glioblastomas' which originate spontaneously in adults. They vary in the mutation spectrum, but have also in common features which may provide a basis for improving the treatment regimen. A common feature of most of the primary glioblastomas is the presence of wild type p53. Human glioblastoma cell lines with wild type p53 primarily activate a sustained growth arrest in response to DNA damaging procedures and show features of senescence. The response is cooperatively controlled by the tumor suppressors p53 and Rb2, and obviously does not require a functional p16INK4a. Cells expressing mutant p53 primarily respond with apoptosis. Thus, the genetic status of *p53* and its interaction with Rb2 may have a severe impact on the outcome of glioblastoma treatment. Understanding the molecular pathways controlling the growth arrest in this response could be a major step forward to adjust chemotherapeutic treatment and to lower the risk of tumour recurrences.

Functional interactions of p53 with telomeres and telomerase

W. Deppert, C. Günes, K. Iwanski, N. Simon, B. Wittek

Telomeres, the end structures of linear chromosoms, are protein-DNA complexes. They function to protect the chromosoms from end-to-end fusions, degradation by nucleases and prevent loss of genetic material during replication. For the complete replication of telomeric DNA, a repetetive sequence at the end of linear chromosoms the activity of telomerase, a cellular RNA-dependent DNA polymerase, is required. Thus, functional telomeres and telomerase contribute to genomic stability. There is accumulating evidence that the tumor supressor protein p53 acts in concert with telomeres and telomerase to warrant the stability of the genome: p53 seems to bind both the telomeric DNA and the telomerase complex, at least *in vitro.* Impaired telomere/telomerase function activates DNA-damage pathways, including the p53 mediated growth arrest or apoptosis. Moreover, over-expression of p53 down-regulates the expression of *TERT,* the catalytic subunit of telomerase. Despite the knowledge about the cooperation of p53 with telomere/telomerase function in maintaining the integrity of the genome, molecular mechanisms how these components act together, is not fully understood yet. We aim to identify the regulatory mechanisms how p53 cooperates with telomere and telomerase functions and under which conditions p53 becomes activated in response to dysfunctional telomeres. Also, it remains to be elucidated yet, under which cellular conditions p53 regulates *TERT* gene expression and telomerase activity.

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The MDM2 ubiquitination signal in the DNA-binding domain of p53 forms a docking site for calcium calmodulin kinase superfamily members

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Genetic and biochemical studies have shown that Ser(20) phosphorylation in the transactivation domain of p53 mediates p300 catalyzed DNA-dependent p53 acetylation and B-cell

tumour suppression. However, the protein kinases that mediate this modification are not well-defined. A cell-free Ser(20) phosphorylation site assay was used to identify a broad range of Calcium-calmodulin kinase superfamily members including CHK2, CHK1, DAPK-1, DAPK-3, DRAK-1, and AMPK as Ser(20) kinases. Phosphorylation of a p53 transactivation domain fragment at Ser(20) by these enzymes in vitro can be mediated in trans by a docking site peptide derived from the BOX-V domain of p53, which also harbours the ubiquitin-signal for MDM2. Evaluation of these Calcium-calmodulin kinase superfamily members as candidate Ser(20) kinases in vivo has shown that only CHK1 or DAPK-1 can stimulate p53 transactivation and induce Ser(20) phosphorylation of p53. Using CHK1 as a prototypical in vivo Ser(20) kinase, we demonstrate that (i) CHK1 protein depletion using siRNA can attenuate p53 phosphorylation at Ser(20), (ii) an EGFP-BOX-V fusion peptide can attenuate Ser(20) phosphorylation of p53 in vivo, (iii) the EGFP-BOX-V fusion peptide can selectively bind to CHK1 in vivo, and (iv) the Deltap53 spliced variant lacking the BOX-V motif is refractory to Ser(20) phosphorylation by CHK1. These data indicate that the BOX-V motif of p53 has evolved the capacity to bind to enzymes that mediate either p53 phosphorylation or ubiquitination thus controlling the specific activity of p53 as a transcription factor.Homeodomain-Interacting Protein Kinase 2 Is the Ionizing Radiation-Activated p53 Serine 46 Kinase and Is Regulated by ATM.

Supported by the Deutsche Forschungsgemeinschaft

Structural heterogeneity of urea-denatured tumor suppressor p53

W. Deppert, J. Heukeshoven, A. März, G. Tolstonog

Human recombinant, baculovirus-expressed p53 protein focuses on 2D-gels in about 30 spots in the pI range of 5.5 – 6.7. Re-electrophoresis of p53 isolated from individual spots resulted in the appearance of multiple spots deviating in pI value from the original one. Furthermore, 2D-gel analysis of RP-HPLC chromatographic fractions showed that p53 from each fraction was again distributed over the whole pH range of 5.5 – 6.7. Okadaic acid treatment of insect cells, phosphate substitution reaction of purified p53 and individual analysis of all spots by mass spectrometry revealed that only a very minor fraction of the baculovirus-expressed p53 molecules is modified by phosphorylation. This finding excluded that the individual p53 spots in 2D-gels reflect charge isomers generated by phosphorylation, but rather suggest that they are due to different structural properties of the urea-denatured monomeric p53 molecule. This structural heterogeneity was neither species-specific, nor characteristic for baculovirus-expressed p53. Moreover, mutant p53 did not deviate from wild-type p53 in this property, indicating that this property is inherent to p53. As deletion of the central DNA-binding domain abrogated focusing of p53 into multiple spots, we conclude that this domain mainly contributes to the observed heterogeneity. Our data demonstrate that 2D gel electrophoresis is not a reliable tool for the identification of charge isomers of proteins. Thus results obtained with this method have to be viewed with caution and verified by additional proteomic approaches.

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II. Novel Mouse Models for Tumor Diagnosis and Treatment

Genetically modified mice as models to analyze the oncogenic potential of p53

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Mutations in the *p53* tumor suppressor gene may not only result in a loss of wild-type (wt) p53 functions, but also in the generation of mutant p53 (mutp53) proteins with gain-of-function properties. The "gain of function" hypothesis for mutant p53 (mutp53) is supported by various in vitro studies, as well as by correlative analyses of tumor patient data showing that expression of mutp53 often is associated with bad prognosis.

In order to analyze the postulated causal relationship between specific mutp53 proteins and mammary carcinoma progression in vivo we generated and characterized inducible BALB/c WAP-mutp53 transgenic mouse models, each carrying a murine, mutant *p53* minigene harboring point mutations equivalent to human tumor derived hot spot mutations (murine mutp53^{R245W} corresponding to human mutp53^{R248W}, murine mutp53^{R270H} corresponding to human mutp53^{R273H}). A WAP-mutp53^{R172H} transgenic mouse line was obtained in a FVB-background and back-crossed into the BALB/c background. In detail we characterized a WAP-mutp53^{R172H}, 2 WAP-mutp53^{R245W} (W1, W10) and 2 WAP-mutp53^{R270H} (H8, H22) transgenic mouse lines. Phenotypic characterizations of these transgenic WAP-mutp53 lines so far did not show a direct impact of any of these mutp53 proteins on mammary carcinoma initiation after a long term observation, but provided hints for a role of mutp53 in tumour progression. It is important to note that mutp53 expression as such did not increase the frequency of mammary tumor development in WAP-mutp53 mice compared to parental BALB/c mice, even in WAP-mutp53 lines that constitutively express high levels of mutp53 in the mammary glands. Histopathological grading indicates a role of mutp53 in mammary carcinoma progression, associated with a higher rate of metastasis. Our data comprise the first in vivo evidence for a gain of function of mutp53 in mammary carcinogenesis in an adult organ. The underlying molecular mechanisms will be analyzed in detail.

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Differential regulation of human and mouse TERT promoter activity during testis development

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Activity of telomerase is a prerequisite for continuous proliferation capacity of stem cells or progenitor cells. Rapid down-regulation of telomerase occurs during differentiation of stem cells pointing out the importance of a tight regulation of telomerase in human tissues. However, its role and the molecular mechanisms underlying telomerase activity in progenitor or stem cells are not well understood. The main reason for this lack of information is the absence of reliable detection methods for the cell type specific expression/activity of telomerase. Several commercially available antibodies exist against *TERT* component. Analyses of human tissues with these antibodies either did not show any *TERT* expression or did not provide a non-ambiguous result.

In adult testis, telomerase activity is exclusively observed during the early steps of spermatogenesis and is downregulated during differentiation to spermatozoa. Knowledge about telomerase activity during testis development from birth to adulthood is still scarce. We used the hTERTp-lacZ transgenic mouse model that expresses the bacterial lacZ reporter gene under the controlof an 8.0-kbp human TERT promoter fragment to analyze simultaneously endogenous mouse TERT gene expression as well as human TERT promoter activity during mouse testis development. We show that human TERT promoter activity is more stringently controled when compared to mouse TERT expression. Mouse TERT expression and telomerase activity were found to be high in testis from the earliest time point tested (6 days post partum), whereas human TERT promoter activity increased during puberty and was highest in adult mouse testis. Histological analysis revealed that β-galactosidase expression, encoded by the lacZ reporter gene, is present in all seminiferous tubules in adult testis, but in a subset of tubules before puberty. Moreover, in prepubertal testes, we observed a spatial and preclusive expression pattern of human TERT promoter activity and activated c-kit.

Supported by Deutsche Krebshilfe

Regulation of telomerase activity during liver regeneration and liver cell differentiation

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Telomerase activity is non detectable in human liver biopsies whereas increased telomerase activity is found during liver carcinogenesis indicating that telomerase activity is repressed during differentiation of human liver and reactivation must occur during carcinogenesis. Here we analysed the regulation of telomerase activity during liver cell proliferation and differentiation in in vitro and in vivo models. As an in vivo model we investigated the regulation of human TERT gene promoter activity during regeneration induced by partial hepatectomy in hTERTp-lacZ transgenic mice which contain an 8.0 kbp fragment of the human TERT gene promoter. As an in vitro model we used HepaRG cell line which recently was established as a new system for human liver cell differentiation. We found a sharp increase of human TERT promoter activity at day 3 after partial hepatectomy whereas murine TERT mRNA expression was only weakly induced at the same time. A weak but significant increase in telomerase activity was observed at day 2 after PH which did not correlate with TERT mRNA levels during regeneration. On the other hand, telomerase activity and TERT gene expression is detectable in proliferating HepaRG cells and is markedly down-regulated as cells reach confluency and cease proliferation. Luciferase reporter gene analyses indicated that the 0.3 kbp TERT promoter fragment posseses all necessary cis regulatory elements for TERT gene repression in HepaRG cells. We established a suitable in vivo and in vitro model to dissect the molecular mechanisms underlying the regulation of telomerase activity in liver cells.

Supported by Deutsche Krebshilfe

Dissecting the mechanism of apoptosis-resistance in hematopoietic malignancies expressing wild-type p53

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Tumors that have acquired resistance against death stimuli constitute a severe problem in the context of cancer therapy. To determine genetic alterations that favor the development of stress resistant tumors *in vivo,* we have taken advantage of polyclonal tumors created by retroviral infection of newborn Eλ-myc mice, in which the retroviral integration acts as a gene mutagen to enhance tumor progression. Tumor cells were exposed to gamma-irradiation prior to their re-transplantation into syngenic recipients, thereby providing a strong selective pressure for pro-survival mutants. Secondary tumors developing from stress-resistant tumor stem cells were analyzed for retroviral integration sites to reveal candidate genes whose dysregulation confer survival. In addition to the anti-apoptotic gene *Bcl-xL,* we identified the *Gadd45β* locus to be a novel common integration site in these stress-resistant tumors. Enhanced expression of Gadd45β was observed in secondary tumors, but not in cells from primary non-stressed parental tumors. In accord with a thus far undocumented supportive role of Gadd45β in tumorigenesis, we showed that Gadd45β over-expressing NIH3T3 cells form tumors in NOD/SCID mice. Interestingly, and in contrast to "classical" anti-apoptotic factors, high Gadd45β levels did not protect against c-myc, UV or gamma-irradiation induced apoptosis, but conferred a strong and specific survival advantage against serum withdrawal. We are currently analyzing additional tumor pairs (radiation sensitive vs. resistant) by DNA microarray technology to identify differentially regulated genes that may contribute to tumor growth and/or therapy-resistance. Three candidate genes preferentially upregulated in the radiation resistant tumors are currently being analyzed.

Supported by Deutsche Krebshilfe

III. Analysis of Key Regulators in Disease

1. Signal Transduction

Homeodomain-interacting protein kinase 2 is the ionizing radiation-activated p53 serine 46 kinase and is regulated by ATM

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Phosphorylation of p53 at Ser(46) is important to activate the apoptotic program. The protein kinase that phosphorylates p53 Ser(46) in response to DNA double-strand breaks is currently unknown. The identification of this kinase is of particular interest because it may contribute to the outcome of cancer therapy. Here, we report that ionizing radiation (IR) provokes homeodomain-interacting protein kinase 2 (HIPK2) accumulation, activation, and complex formation with p53. IR-induced HIPK2 up-regulation strictly correlates with p53 Ser(46) phosphorylation. Down-regulation of HIPK2 by RNA interference specifically inhibits IR-induced phosphorylation of p53 at Ser(46). Moreover, we show that HIPK2 activation after IR is regulated by the DNA damage checkpoint kinase ataxia telangiectasia mutated (ATM). Cells from ataxia telangiectasia patients show defects in HIPK2 accumulation. Concordantly, IR-induced HIPK2 accumulation is blocked by pharmacologic inhibition of ATM. Furthermore, ATM down-regulation by RNA interference inhibited IR-induced HIPK2 accumulation, whereas checkpoint kinase 2 deficiency showed no effect. Taken together, our findings indicate that HIPK2 is the IR-activated p53 Ser(46) kinase and is regulated by ATM.

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Analysis of nucleocytoplasmic trafficking of the HuR ligand APRIL and its influence on CD83 expression

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Dendritic cells (DC) are the most potent antigen-presenting cells of the immune system and are able to sensitize even naïve T cells. Mature DC are characterized by expression of CD83, a surface molecule that is proposed to be involved in efficient T cell activation. It has been recently shown that CD83 mRNA is transported from the nucleus to the cytoplasm in a HuR- and CRM1-dependent manner. Therefore we here investigated the impact of two known protein ligands of HuR, pp32 and APRIL, on CD83 expression. Both pp32 (ANP32A) and APRIL (ANP32B) are shuttle proteins and it has been reported earlier that these HuR-ligands can act as adaptors that link HuR and the CRM1-specific nuclear export pathway. By employing RNA interference (RNAi) technology we demonstrated that pp32 is dispensable for CD83 expression, whereas APRIL contributes to the nuclear export and subsequent translation of CD83 mRNA. Furthermore, we have determined the nuclear import signal (NLS) as well as the nuclear export signal (NES) of human APRIL. Moreover, we analyzed the status of phosphorylation of endogenous APRIL and identified Thr^{244} to be a so far unrecognized phosphate acceptor. Finally, we were able to show that phosphorylation of this specific amino acid residue regulates the nuclear export of APRIL. In sum, we identified the signal sequences in APRIL that mediate its intracellular trafficking and provided evidence that this protein ligand of HuR is an important player in the posttranscriptional regulation of CD83 expression by affecting the nucleocytoplasmic translocation of CD83 mRNA. Our combined results point to an important role of the CRM1-dependent nuclear export pathway for the expression of CD83 under conditions of T cell activation and DC maturation. Therefore, inhibition of this specific nuclear export pathway provides a novel strategy for immune-modulation therapy.

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G protein coupled receptors. Cell surface receptors and disease development

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Traditionally, G protein-coupled receptors were thought to act as monomers, in which one ligand binds and activates a single receptor-G protein complex. However, our data and those published by others suggest that a dimer is the minimal functional structure. Moreover,

considerable variation may exist on effects elicited by agonists on the quaternary structure. For instance, the AT_1 receptor forms heterodimers with various other receptors such as the bradykinin B_2 receptor, the AT_2 receptor, the 2-adrenergic receptor, and the dopamine D1 receptor. Therefore, still ongoing work deals with pathophysiological relevance of GPCR dimerization and oligomerization *in vivo*.

We previously detected increased levels of AT_1/B_2 receptor heterodimers on platelets and vessels of preeclamptic women. Preeclampsia is a pregnancy-specific hypertensive disorder with unknown etiology, which affects 5% to 10% of all pregnancies. This disease is characterized by a rapidly progressive rise in blood pressure and the presence of protein in the urine. Our data revealed increased levels of heterodimers between the vasopressor receptor AT_1 and the vasodepressor receptor B_2. We concluded that a major symptom of preeclampsia is the result of complex formation between two G-protein-coupled receptors. Enhanced angiotensin II responsiveness is also a prominent feature of essential hypertension and of related cardiovascular disorders. Remarkably, hypertensive patients display an enhanced angiotensin II-dependent monocyte activation and adhesion to endothelial cells. We assumed that enhanced monocyte adhesiveness sensitizes hypertensive patients to the development of atherosclerosis and that the nature and extent of the inflammatory response within an atherosclerotic lesion contribute to its susceptibility to rupture, which can in turn initiate local thrombosis and the ensuing complications of acute myocardial infarction and stroke.

We found that intracellular factor XIIIA transglutaminase crosslinks agonist-induced AT_1 receptor homodimers via glutamine^{315} in the carboxyl-terminal tail of the AT_1 receptor. The crosslinked dimers displayed enhanced signaling and desensitization *in vitro* and *in vivo*. Inhibition of angiotensin II release or of factor XIIIA activity prevented formation of crosslinked AT_1 receptor dimers. In agreement with this finding, factor XIIIA-deficient individuals lacked crosslinked AT_1 dimers. Elevated levels of crosslinked AT_1 dimers were present on monocytes of patients with the common atherogenic risk factor hypertension and correlated with an enhanced angiotensin II-dependent monocyte adhesion to endothelial cells. Because inhibition of angiotensin II generation or of intracellular factor XIIIA activity suppressed the appearance of crosslinked AT_1 receptors and symptoms of atherosclerosis in ApoE-deficient mice, we assume that elevated levels of crosslinked AT_1 receptor dimers on monocytes sustain the process of atherogenesis. The dynamic interplay between constitutive GPCR clustering, ligand-induced receptor rearrangements and specific biological or pathological outcomes will be a significant avenue of future investigation.

Disturbed crosstalk between growth factor and glutamate triggered ionotropic calcium channels defines aggressive acute leukemic cells

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Blood cell development depends on signal transduction cascades triggered by lineage specific growth factors, serum and medium components. These cascades regulate growth, differentiation and apoptotic cell death but are disturbed by inherited or acquired genetic defects. Since stringently regulated calcium concentrations are required for differentiation, growth and apoptosis, we suspected that calcium import is disturbed in autonomous growing cell clones due to deregulated channel gene expression. We established growth factor-independent cell clones by cocultivation of myeloid progenitor TF1 cells with vector producing fibroblasts and screened proviral integrations for tagged calcium channel genes. This approach makes use of gene tagging that allows identifying genes involved in tumor development. We identified GluR5 as a tagged gene, which is a subunit of glutamate/kainate responsive, ionotropic plasma membrane Ca^{2+}/Na^{+} channels comprising five members (GluR5-7, KA1-2). These channels are involved in neuronal signal transmission, neuronal development and neuronal diseases. Since these channels were found even in plant cells, and are activated by glutamate, it is indicated that they are involved in various cellular aspects of non-neuronal cells as well. We found GluR5 repression in growth factor-dependent cell lines, expression in human $CD34^{+}$ bone marrow and peripheral blood cells. We found that this calcium channel supports apoptosis. Stroma cells block apoptosis and support myeloid cell proliferation but allow aberrant GluR5 splicing in genetically impaired cells and outgrowth of autonomous cell clones. An aberrant splice variant identified in growth factor-independent cell lines and leukemic blasts blocks blood cell development by heterodimer formation and uncouples internal calcium pools from signal transduction supporting apoptosis in response to growth factor starvation depending on initial genetic defects. We found moreover constitutive expression of second ionotrophic calcium channel. This channel imports low calcium influx and is required for cell growth. This finding explains the absolute requirement for glutamate for any cell and allows blocking *ex vitro* and *in vivo* tumor cell growth. We are currently involved in setting vectors to confirm this finding and to test whether this channel forms homomeric and or heterodimeric or multimeric channel complexes. Moreover, we started to establish purging protocols and screen for suitable antagonists that block invasive tumor development.

2. Nuclear Architecture

FLASH meets nuclear bodies: CD95 receptor signals via a nuclear pathway

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The CD95 receptor signals via assembly of a multi-protein complex termed death-inducing signaling complex (DISC) which triggers activation of receptor-bound caspase-8/FLICE molecules. Most cells (type II cells) depend on a mitochondrial amplification pathway to commit apoptosis upon CD95 activation. The caspase-8-binding protein FLICE-associated huge protein (FLASH) has been previously implicated in the regulation of caspase-8 activation at the DISC. However, recent findings demonstrated that FLASH is a Cajal body component and regulates progression through S-phase of the cell cycle in the nucleus. Our recent work identified FLASH as binding partner of the PML nuclear body (PML NB) constituent Sp100 and demonstrated that FLASH partially localizes to PML NBs. Upon CD95 activation FLASH exits the nucleus and translocates to mitochondria where it meets caspase-8 to promote its activation. Our findings reconcile conflicting views on FLASH localization and its role in apoptosis regulation, and suggest that CD95 signals via a nuclear pathway. Potential implications of our findings for understanding FLASH function are discussed.

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A novel chromatin condensing nuclear protein with implications for a role in mitotic chromosome condensation

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The protein elements responsible for higher ordered compaction of chromatin into condensed mitotic chromosomes, still to date remain poorly understood. In this paper we show for the first time that a novel PHD containing protein called SPOC1 is chromatin associated, highly labile and is capable of inducing massive global chromosome condensation. SPOC1 is regulated in both its localization and expression levels during the cell cycle. At the G1/S-phase transition SPOC1 is released from the relaxed chromatin until late S-phase/G2 when it becomes reloaded again. SPOC1 protein levels gradually increase from mid-late S-phase and climax during mitosis. Consistent with a potential role in chromatin modulation/condensation, SPOC1 demonstrates a regulated and bi-phasic localization to condensed mitotic chromosomes and its siRNA mediated reduction results in impaired mitotic chromosome condensation. Taken together we have discovered a novel and dynamic player in chromosome condensation and cell division.

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3. Viral oncogenes

Adenovirus early region 1B 156R protein promotes cell transformation independent from repression of p53-stimulated transcription

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Early region 1B (E1B) of adenovirus type 5 (Ad5) encodes at least five different polypeptides generated by alternative splicing of a common mRNA precursor. Two of these gene products, E1B-19K and E1B-55K, are individually capable of cooperating with the Ad5 E1A proteins to completely transform rodent cells in culture. Substantial evidence suggests that these two E1B proteins contribute to cell transformation by antagonizing growth arrest and apoptosis. Here, we performed genetic and biochemical analyses to assess the attributes of the remaining E1B proteins (E1B-156R, E1B-93R and E1B-84R). Our results show that E1B-156R, which comprises the 79 amino-terminal and 77 carboxy-terminal amino acids of E1B-55K, also enhances focal transformation of primary rat cells in cooperation with E1A. Since E1B-156R seemed unable to relocalize p53 and inhibit its transactivating function, it must be assumed that it contributes to transformation independently of repression of p53-stimulated transcription. Furthermore, we discovered that E1B-156R contains a functional transcriptional repression domain and binds Ad5 E4orf6 and the cellular apoptosis regulator Daxx. While the ability to bind E4orf6 could indicate further biological functions of E1B-156R in viral infection, the interaction with Daxx might also be linked to its transforming potential. Taken together, these analyses introduce E1B-156R as a novel transformation-promoting E1B protein that acts

without repressing p53-transactivation. Moreover, identification of the interaction partners E4orf6 and Daxx provide a first glance of E1B-156R's potential functions.

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The adenovirus E1B-55K oncoprotein induces SUMO modification of p53

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The adenovirus E1B-55K oncoprotein exerts several activities, including the induction of ubiquitination, to interfere with the function of p53 and other host cell proteins. Here we unravel a novel activity of E1B-55K by demonstrating that it drastically stimulates the post-translational modification of p53 by the ubiquitin-like SUMO modifier. Consistent with this finding the extent of p53 SUMOylation is highly elevated in adenovirus transformed cell lines. E1B-55K-mediated SUMOylation depends on the direct interaction of E1B-55K with p53 and additionally requires SUMO modification of E1B-55K. These data indicate that E1B-55K exploits both ubiquitin and ubiquitin-like systems to target host cell proteins and thus shed new light on the molecular mechanisms of E1B-55K function.

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Mechanisms independent from blocking p53-activated transcription contribute to cell transformation by the adenovirus type 5 early region 1B 55-kDa oncoprotein

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Inhibition of p53-activated transcription is an integral part of the mechanism by which early region 1B 55K oncoprotein (E1B-55K) from adenovirus type 5 (Ad5) contributes to complete cell transformation in combination with Ad E1A. In addition, more recent data suggest that the mode of action of the Ad protein during transformation may involve additional functions and other protein interactions. In the present study we performed a comprehensive mutational analysis to assign further transforming functions of Ad5 E1B-55K to distinct domains within the viral polypeptide. Results from these studies show that the functions required for transformation are encoded within several patches of the 55K primary sequence including several clustered cysteine and histidine residues, some of which match the consensus for zinc fingers. In addition, two amino acid substitutions (C454S/C456S) created a 55K mutant protein which was substantially reduced in transforming activity. Interestingly, the same mutations did neither affect binding to p53 nor inhibition of p53-mediated transactivation. Therefore, an activity necessary for efficient transformation of primary rat cells can be separated from functions required for inhibition of p53-stimulated transcription. Our data indicate that this activity is linked to the ability of the Ad5 protein to bind to components of the Mre11/Rad50/NBS1 DNA double-strand break repair complex and/or its ability to assemble

multiprotein aggregates in the cytoplasm and nucleus of transformed rat cells. These results introduce a new function for Ad5 E1B-55K and suggest that the viral protein contributes to cell transformation through p53 transcription-dependent and -independent pathways.

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Function of the adenovirus E1B-55K oncoprotein is modulated by minor E1B products

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The adenovirus E1B-55K protein has several functions required for optimal virus replication and complete cell transformation. It is generally considered that these activities are due to 55K's ability to counteract p53 tumor suppressor function. At least three additional proteins termed E1B-156R, -93R and -84R are produced from alternatively spliced E1B mRNAs. These minor E1B proteins all share the N-terminus with E1B-55K but differ in the C-terminal sequences. Although their role in lytic infection and cell transformation is still unclear it has been speculated that they may regulate the activity of E1B-55K. We, therefore, investigated possible interactions between the E1B proteins. Our results show that E1B-156R and -93R form homodimers through the N-terminal 79 amino acids present in both E1B proteins. We further demonstrate that E1B-156R and -93R interact with E1B-55K, presumably by formation of heterodimers and show that E1B-156R relieves transcriptional repression of p53 by E1B-55K. These data indicate that the multifunctional properties of E1B-55K are regulated through protein-protein interactions with smaller E1B products.

RNA interference as a tool to study adenovirus E1A/ E1B-mediated cell transformation

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The large E1B proteins (E1B-55K) from human subgroup A and C adenoviruses (Ads) are capable of cooperating with Ad E1A to transform primary mammalian cells in culture. It is generally considered that 55K products contribute to complete cell transformation by antagonizing apoptosis and growth arrest, which primarily result from the induction and metabolic stabilization of the tumor suppressor protein p53 by Ad E1A. These growth-promoting activities correlate with its ability to act as a direct transcriptional repressor that is targeted to p53-responsive promoters by binding to p53. In addition it has been hypothesized that the mode of action of Ad E1B-55K products during transformation may involve additional functions and other protein interactions. In the present study we evaluated the use of RNA interference (RNAi) as an experimental tool to assess the role of known and potential E1B-55K interaction partners on E1A- plus E1B-mediated transformation of primary baby rat kidney (BRK) cells. Results from these studies demonstrate that coexpression of E1A with p53-specific short hairpin RNAs (shRNAs) efficiently induced the formation of transformed clones which could be established into permanent cell lines. Surprisingly, shRNA-p53 appeared to be more efficient to promote E1A-induced focus formation than E1B-55K. More importantly, the total number of transformed cells was further greatly increased when both plasmids were simultaneously cotransfected with E1A, suggesting that E1B-55K and shRNA-p53 can synergistically promote focus-formation in the presence of E1A. These data demonstrate that silencing of cellular targets of E1B-55K through RNAi can be used to evaluate their role in cell transformation and provide further support for the idea that the viral oncoprotein contributes to oncogenesis through p53-dependent and -independent pathways.

Tumorigenic potential of gammaherpesvirus-encoded miRNAs

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The etiology of several tumors of B cell, endothelial and epithelial origin (e.g., Burkitt's lymphoma, primary effusion lymphoma, Kaposi's sarcoma) is strongly linked to infection with the gammaherpesviruses Epstein-Barr Virus (EBV) and Kaposi's sarcoma-associated herpesvirus (KSHV). In all these malignancies, the tumor cells are latently infected with EBV or KSHV, and it is consequently thought that the products latency genes play a major role in the onset and/or progression of tumorigenesis. While much attention has been focused on the tumorigenic potential of latency proteins, we have recently identified a number of microRNAs (miRNAs) which are encoded by KSHV and EBV and which are expressed during the latent phase of infection. Given the ability of miRNAs to modulate cellular gene expression and their frequent deregulation in human cancers, we hypothesize that viral miRNAs might contribute to the development of KSHV- and EBV-associated malignancies. To test this hypothesis, we have generated recombinant retroviruses which express various combinations of viral miRNAs. We are currently investigating the potential of miRNA-expressing mouse embryonic stem cells to give rise to tumors in a mouse model of Burkitt's lymphoma (i.e., mice which overexpress the c-myc proto-oncogene). Since it is possible that viral miRNAs co-operate with proteinaceous components of the latency program to induce cellular transformation, we are also creating vehicles which allow co-expression of viral miRNAs and viral latency proteins. These experiments will establish whether virally encoded miRNAs are likely to be involved in the development of herpesvirus-associated cancers and, if so, which of the miRNAs (or combination of miRNAs) are the key players driving the pathogenic processes which induce or augment cellular transformation.

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4. Transcriptional and Posttranscriptional Regulation

The RUNX1 transcription factor: A gatekeeper in acute leukaemia

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RUNX1, encoding a heterodimeric transcription factor, is one of the most frequently mutated genes in human acute leukemias and hematopoietic disorders. Located on chromosome 21, it is directly targeted by several chromosomal translocations in AML, with the t(8;21) being the most common. Moreover, 20% of pediatric B-cell acute lymphoblastic leukemia (ALL) carry the t(12;21), juxtaposing the *RUNX1* gene with the gene encoding the ETS transcription factor TEL. In addition to translocations, inactivating or dominant-negative mutations in the

RUNX1 gene have been identified in 15 to 25% of the relatively rare, minimally differentiated Mo-AMLs and up to 25% of myelodysplastic syndromes associated with AML development. We have established mouse models for translocations t(8;21) and t(12;21) and, more recently, for mutated *RUNX1* genes, using a retroviral-vector transduction protocol coupled with bone marrow transplantation. Our sytem allows the assessment of changes in the hematopoietic system before the onset of an overt leukemia, providing an excellent model to study the mechanism. The evaluation of these mice have lead to the following conclusions: 1) intrinsic functions of the fusion protein or mutated protein play a determining role in the phenotype of the ensuing leukemia; 2) whereas repression of direct target genes of RUNX1 is a common functional denominator of RUNX1 fusion proteins, DNA-binding-independent functions of RUNX1 may play an important role in leukemogenesis; 3) RUNX1 gene alterations alone are insufficient to induce a leukemia, but probably act as gatekeeper, permitting the outgrowth of a preleukemic clone, which is the target of secondary events. We are currently extending our work to human hematopoietic cells, using a xenograft transplantation model. For these studies we have developed lentiviral vectors that allow the efficient expression of a fluorescent marker gene (e.g. VENUS, tdTomato, EGFP, or Cyan), a potential oncogene, and a cassette to express short-hairpin RNA, suppressing gene expression of a potential tumor suppressor. Finally, in collaboration with the Kinderkrebs-Zentrum (Prof. Martin Horstmann), we are testing candidate genes that may synergize with RUNX1 mutations to induce an acute leukemia.

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The MADS transcription factor MEF2C is an important regulator of monopoiesis and the leukemic stem cell compartment in MLL-associated leukemia

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The MEF2 proteins are members of the MADS (MCM1-agamous-deficiens-serum response factor) family of transcription factors, which play key roles in pattern development in plants and coordinate diverse and important biological functions, including proliferation, differentiation, and apoptosis, in the animal. The MEF2 subfamily was originally characterized as a sequence-specific DNA binding activity of various genes encoding muscle structural proteins and has since been shown to be important in neuronal development. We identified the gene in a "retroviral insertional mutagenesis" strategy to identify novel genes in the induction of acute leukemia. Using a retroviral gene transduction – bone marrow transplantation model we have been able to demonstrate the importance of MEF2C in AML development in susceptible mice. This analysis has also shown the importance of MEF2C in lineage commitment to the monocytic lineage, which has been corroborated by analysis of conditional *Mef2c* knock-out mice. Analysis of AML patient samples provided the insight that high MEF2C expression is found in myelomonocytic leukemia samples characterized by alterations of the *bithorax* orthologue MLL. Deletion of the *Mef2c* gene in MLL-transformed cells cripples their ability to induce leukemia in vivo and thus may play an important role in maintaining the LSC compartment.

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Hypusination of eukaryotic initiation factor 5A (eIF-5A): A novel therapeutic target in BCR-ABL positive leukemias identified by a global proteomics approach

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Inhibition of *BCR-ABL* tyrosine kinase with Imatinib represents a major breakthrough in the treatment of patients with chronic myeloid leukemia (CML). However, resistance to Imatinib develops frequently, particularly in late stage disease. To identify new cellular *BCR-ABL* downstream targets, we analyzed differences in global protein expression in *BCR-ABL*-positive K562 cells treated with or without Imatinib in vitro. Among the 19 proteins found to be differentially expressed, we detected the down-regulation of eukaryotic initiation factor 5A (eIF-5A), a protein essential for cell proliferation. EIF-5A represents the only known eukaryotic protein activated by post-translational hypusine-modification. Hypusine-modification inhibitors (HI) alone exerted an anti-proliferative effect on *BCR-ABL*-positive and -negative leukaemia cell lines in vitro. However, the synergistic dose-response relationship found for the combination of Imatinib and HI was restricted to *BCR-ABL*-positive cells. Furthermore, this synergistic effect was confirmed by cytotoxicity assays, cell cycle analysis and CFSE-labeling of primary $CD34^+$ CML cells. Specificity of this effect could be demonstrated by co-treatment of K652 cells with Imatinib and short hairpin RNA (shRNA) against eIF-5A, which were expressed by use of lentiviral pseudotypes. In conclusion, through a comparative proteomics approach and further functional analysis, we identified hypusine-modification of eIF-5A as a promising new approach for combination therapy in *BCR-ABL* positive leukemias.

Sumoylation in axons triggers retrograde transport of the RNA-binding protein La

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A surprisingly large population of mRNAs has been shown to localize to sensory axons, but few RNA-binding proteins have been detected in these axons. These axonal mRNAs include several potential binding targets for the La RNA chaperone protein. La is transported into axonal processes in both culture and peripheral nerve. Interestingly, La is posttranslationally modified in sensory neurons by sumoylation. In axons, small ubiquitin-like modifying polypeptides (SUMO)-La interacts with dynein, whereas native La interacts with kinesin. Lysine 41 is required for sumoylation, and sumoylation-incompetent La(K41R) shows only anterograde transport, whereas WT La shows both anterograde and retrograde transport in axons. Thus, sumoylation of La determines the directionality of its transport within the axonal compartment, with SUMO-La likely recycling to the cell bod.

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5. Modifiers

Modification of eukaryotic initiation factor 5A from Plasmodium Vivax by a truncated deoxyhypusine synthase from Plasmodium Falciparum: An enzyme with dual enzymatic properties

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The increasing resistance of the malaria parasites enforces alternative directions in finding new drug targets. Present findings from the malaria parasite *Plasmodium vivax,* causing tertiary malaria, suggest eukaryotic initiation factor 5A (eIF-5A) to be a promising target for the treatment of malaria. Previously we presented the 162 amino acid sequence of eIF-5A from *Plasmodium vivax*. In the present study, we have expressed and purified the 20 kDa protein performed by one-step Nickel chelate chromatography. In Western blot experiments eIF-5A from *P.vivax* crossreacts with a polyclonal anti-eIF-5A anti-serum from the plant *Nicotiana plumbaginifolia (Solanaceae)*. Transcription of eIF-5A can be observed in both different developmental stages of the parasite being prominent in trophozoites. We recently published the nucleic acid sequence from the gnomic clone of P. falciparum strain NF54 encoding a putative deoxyhypusine synthase (DHS), an enzyme that catalyzes the post-translational modification of eIF-5A. After removal of 22 amino acids DHS was expressed as a Histidin fusion protein and purified by Nickel affinity chromatography. Truncated DHS from *P.falciparum* modifies eIF-5A from *P.vivax*. DHS from *P.falciparum* NF54 is a bi-functional protein with dual enzymatic specificities, that is, DHS activity and homospermidine synthase activity (HSS) (0.047 pkatal/mg protein) like in other eukaryotes. Inhibiton of DHS from *P.falciparum* resulted in a K_i of 0.1 microM for the inhibitor GC7 being 2000-fold less active than the nonguanylated derivative 1,7-diaminoheptane. *Dhs* transcription occurs in both developmental stages suggesting its necessity in cell proliferation.

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IV. Cell physiology at the ultrastructure level

Qualitative and quantitative image analysis of ultra structural changes in sarcomere assembly of cMyBP-C knock-in mice

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Mutations in the *MYBPC3* gene encoding cardiac myosin-binding protein C (cMyBPC) are frequent causes of familial hypertrophic cardiomyopathy (FHC), but the mechanisms leading from mutations to disease remain elusive. The recently developed first targeted cMyBP-C knock-in mouse carries a human *MYBPC3* point mutation. It is a G>A transition on the last nucleotide of exon 6, which is expected to result either in exon skipping and premature stop or production of a full-length, missense protein or both. The homozygotes exhibited myocyte and left ventricular hypertrophy with reduced fractional shortening and interstitial fibrosis, the heterozygotes developed only mild myocyte hypertrophy. In the homozygotes, the amount of missense cMyBP-C was 90% lower and the expected truncated protein was undetectable, even after inhibiting the proteasome with MG132. Both missense and nonsense mRNAs were present, but the level of total cMyBP-C mRNA was markedly lower. Inhibition of nonsense-mediated mRNA decay in cultured cardiac myocytes or *in vivo* with cycloheximide or emetine increased the level of nonsense mRNA severalfold. Electron microscopy analysis has shown a correct sarcomere ultrastructure but slightly different distances between the sarcomere bands in the homozygotes. In conclusion, these data indicate that the expression of the point mutation in the whole animal is regulated mainly at the mRNA level, involving nonsense-mediated mRNA decay. These data contribute to an improved understanding of the molecular mechanisms of FHC.

Electron microscopical dissection of the exoerythrocytic forms of Plasmodium berghei in situ

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The initiation of clinical malaria evolves after the schizogonic division of the exoerythrocytic forms (EEFs) of the parasite and the formation of merozoites inside host hepatocytes. However, the mechanism by which hepatic merozoites reach blood vessels (sinusoids) in the liver and escape the host immune system before invading erythrocytes still remains unknown. Recently it was shown that parasites induce the death and the detachment of their host hepatocytes, followed by the budding of parasite-filled vesicles (merosomes) into the sinusoid lumen. Parasites simultaneously inhibit the exposure of phosphatidylserine on the outer leaflet of host plasma membranes, which act as “eat me“ signals to phagocytes. Thus, the hepatocyte-derived merosomes appear to ensure both the migration of parasites into the bloodstream and their protection from the host immune system. With the help of specially adapted correlative light / electron microscopy methods and novel pre-selection and micro-preparation techniques we were able to localize and to make a pin-point dissection of EEF’s *in situ* in the liver.

Ultrastructural investigation of the in vitro life cycle of Schistosoma mansoni, analysed by cryo-techniques and TEM-tomography, inclusive the localisation of specific regulative proteins in situ.

H. Haas (1), H. Hohenberg (1), B. Holstermann (1), R. Reimer (1), C. Schneider (1), G. Schramm (2), M. Warmer (1)
(1) Heinrich-Pette-Institute, Hamburg
(2) Research Center Borstel

S. mansoni affects 200 million people worldwide, often with serious consequences. This cooperation project between HPI and FZB will provide a transparent model for studying host/parasite interaction and parasite development (a.o. drug testing) and for obtaining parasite immunomodulatory key molecules. Moreover, it represents an alternative to animal experiments.

The various life cycle stages of *S. mansoni* differ profoundly in their effects on the host. Since access to individual stages for immunobiological studies is hampered during *in vivo* infection, *in vitro* culture of the parasite is an easy-access alternative. Moreover, only our ultrafast cryo-fixation and preparation methods can arrest the different states of infectious eggs. Preserving the life-like state of the parasite we are able to study the time course of expression of individual immunomodulatory egg molecules by 3D ultrastructural investigations combined with the pinpoint localisation of these molecules by immunocytological studies.

ESEM study of maxillary sinus mucosa after surgical treatment for sinus floor elevation.

K.U. Benner (2), U. Genter (2), H. Hohenberg (1), M. Warmer (1)
(1) Heinrich-Pette-Institute, Hamburg
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The most common complication in sinus floor lifting is sinusitis often caused by perforation of the Schneider’s membrane, which is frequently very thin and fragile. This surgical technique requires an elevation of the maxillary sinus mucosa leading to a specific rupture of this multilayer tissue. In our animal model study the still hydrated mucosa and the adjacent compact bone were investigated in the Environmental Scanning Microscope close to ambient pressure. To avoid formation of tissue dehydration artefacts a special gel matrix was used and the pressures as well as the temperatures were carefully controlled in the course of investigation. After comparing several staining procedures Ruthenium Red proved to be the most suitable stain for comparative light and electron microscopic analysis of the different tissue layers and allowed us for the first time to image and analyse possible structural lesions of this commonly applied surgical technique *in situ* and free of dehydration artefacts.

Pathways of nanoparticle-functionalized liposomes in tissues detected by combined magnetic resonance imaging (MRI) and high resolution environmental scanning- and transmission electron microscopy (ESEM, TEM): from animal organism to molecules.

G. Adam (3), U. Beisiegel (4), O.T. Bruns (4), S. Förster (2), H. Hohenberg (1), R. Reimer (1), H. Weller (2)
(1) Heinrich-Pette-Institute, Hamburg
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(4) Department of Biochemistry and Molecular Biology II, University Medical Center Hamburg-Eppendorf

Magnetic nanoparticles became more and more important for applications in biotechnology and biomedicine over the past few years. Examples are magnetothermal therapy or *in vivo* imaging. In our interdisciplinary project we used superparamagnetic manganese ferrite nanocrystals ($MnFe_2O_4$) as contrast agents in Magnetic Resonance Imaging (MRI). Superparamagnetic nanocrystals of different sizes were synthesized, embedded into an amphiphilic polymer shell and embedded into large micelles formed by lipids and injected into mice. After MRI-detection of accumulated nanoparticle-functionalized liposomes inside of the mouse liver, the detected regions were removed by a high speed biopsy gun equipped with a high speed fine-needle. Thereafter the hydrated tissue biopsies were directly transferred as a whole into the wet-ESEM for a further preselection step in order to pinpoint detect tissue areas with nanomarkers. Selected areas were well-directed prepared by micromanipulation methods and processed for imaging analysis at molecular resolution in the TEM, in order to detect the nanomarkers *in situ*.

In summary, the combination of correlative MRI, ESEM and TEM technology enables the distribution and density analysis of functionalysed nanomarkers, injected into living animals and thereafter detected in their organs and exact selected tissue, on the molecular level: a new imaging chain from animal organism to molecules and in principle transferable to the investigation of human tissue.

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Diplomarbeiten, Dissertationen, Habilitationen

Diplomarbeiten

Bohlmann, Mark: Identifizierung und Charakterisierung neuer aviärer Hepatitis B-Virusisolate. Diplomarbeit, Universität Heidelberg, Fakultät für Biowissenschaften.

Gießelmann, Kathrin: Funktionelle Analyse des zellulären Proteins OTT/RBM15. Diplomarbeit, Universität Heidelberg, Fakultät für Biologie.

Hildebrand, Janosch: Regulation der LINE-1 Expression in humanen Tumorzelllinien durch Wildtyp und mutiertes p53. Diplomarbeit, Universität Hamburg, Fakultät für Mathematik, Informatik und Naturwissenschaften.

Jahn, Mike: Beeinflussung der Expression eine Reportergens durch promoterflankierende transposable bzw. repetitive Sequenzen in einem episomalen Vektorsystem. Diplomarbeit, Universität Münster, Fachbereich Biologie.

Knauer, Tanja: Charakterisierung nichtkodierender Transkripte in humanen Glioblastoma Zellen. Diplomarbeit, Universität Erlangen, Fachbereich Medizin.

Kömm, Natascha: Analyse der Funktionen von Rb2/p130 und pRb/p105 in der Induktion zellulärer Seneszenz mittels RNA Interferenz. Diplomarbeit, Universität Hamburg, Fakultät für Mathematik, Informatik und Naturwissenschaften.

Peters, Christian: Untersuchungen zur Funktion der Ubiquitin-spezifischen Protease USP7 im lytischen Infektionszyklus humaner Adenoviren. Diplomarbeit, Universität Hamburg, Fakultät für Mathematik, Informatik und Naturwissenschaften.

Pieper, Dorothea: Untersuchungen zur adaptiven zellulären Immunantwort am Beispiel der HIV/HCV-Coinfektion. Diplomarbeit, Universität Hamburg, Fakultät für Mathematik, Informatik und Naturwissenschaften.

Saker, Jarob: Regulation und Funktion der Telomerase und der Telomerfaktoren während der Differenzierung und Proliferation. Diplomarbeit, Universität Hamburg, Fakultät für Mathematik, Informatik und Naturwissenschaften.

Schaub, Theres: Analyse der Funktion der PEST-Domänen und der GSK-3beta vermittelten Phosphorylierung für die Stabilität des SPOC1-Proteins, Universität Rostock, Mathematisch Naturwissenschaftlicher Fachbereich; Institut für Biowissenschaften.

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Wimmer, Peter: Analyse der transformierenden Eigenschaften des E1B-55K-Proteins von Adenovirus Typ 5. Diplomarbeit, Universität Regensburg, Naturwissenschaftl. Fakultät.

Dissertationen

Friese, Olaf: Ein Mausmodell für die fehlende Erkennung methylierter DNA durch das Mdb1-Protein (Methyl-CpG-bindendes Protein 1). Dissertation, Universität Hamburg, Fakultät für Mathematik, Informatik und Naturwissenschaften, Department Chemie.

Iwanski, Gabriela: „Untersuchungen zur Rolle des Transkriptionsfaktors C/EBPalpha bei der normalen und aberranten Hämatopoese mit Hilfe der RNA-Interferenz Methode“. Dissertation, Universität Hamburg, Fakultät für Medizin.

Kinkley, Sarah: SPOC1: Characterization of a Novel Nuclear Protein with Implication for a Functional Role in Mitotic Chromosome Condensation. Universität Hamburg, Fakultät für Mathematik, Informatik und Naturwissenschaften, Department Biologie.

Kriegs, Malte: Untersuchung des Einflusses des Nicht-Strukturproteins 5A des Hepatitis C Virus auf Zellwachstum, Apoptose und Karzinogese *in vitro* und *in vivo,* Freie Universität Berlin, Fachbereich Biologie, Chemie, Pharmazie.

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Mhamdi, Mouna: Characterization of the envelope-mediated steps in the life cycle of hepatitis B viruses. Universität Hamburg, Fakultät für Mathematik, Informatik und Naturwissenschaften, Department Biologie.

Riu-Garcia, Ana: Functional Analysis of Hepatitis B Virus with Mutations in the Envelope Proteins. Dissertation, Universität Hamburg, Fakultät für Mathematik, Informatik und Naturwissenschaften, Department Chemie.

Sander, Marie-Sophie: Quantifizierung der viralen DNA-Synthese im produktiven Infektionszyklus humaner Adenoviren durch Echtzeit-PCR. Dissertation, Universität Regensburg, Fachbereich Biologie.

Wittek, Britta: Regulation der Telomerase in Mammakarzinomen eines bitransgenen Mausmodells [*Mus musculus* (Linnaeus, 1758)]. Dissertation, Universität Hamburg, Fakultät für Mathematik, Informatik und Naturwissenschaften, Department Biologie.

Habilitationen

Dandri, Dr. Maura: Chronic HBV infection and hepatocarcinogenesis – Lessons from in vitro and in vivo models. Universität Hamburg, Fakultät für Medizin.

Günes, Dr. Cagatay: Regulation und Funktion der Telomerase in normalen und Tumorzellen: Erkenntnisse aus in vitro und in vivo Modellen. Universität Hamburg, Fakultät für Medizin.

Die Kaufmännische Abteilung

Leitung: Dr. Volker Uhl

Personal

Die Personalabrechnung wurde 2007 an einen externen Dienstleister ausgegliedert. Dadurch konnte eine Stelle eingespart und das Qualitätsniveau der Lohn- und Gehaltsabrechnung verbessert werden. Einstellungen, Entlassungen und die gesamte Personalverwaltung werden schneller und dienstleistungsorientierter realisiert.

Finanz- und Rechnungswesen

Der Datentransfer zwischen der Personalabrechnung und dem Buchhaltungssystem konnte automatisiert werden, sodass eine erhebliche Arbeitsverbesserung erreicht wurde. Des Weiteren hat die Arbeitsgruppe Finanz- und Rechnungswesen in 2007 die Reisekostenabrechnung als Aufgabe von der Personalabteilung übernommen: Die Prüfung, Abrechnung und Verbuchung von Dienstreisen wird künftig in einer Person gebündelt. Darüber hinaus wurde das Projekt eines barcodebasierten Anlageinventarisierungsprogrammes in 2007 erfolgreich fortentwickelt.

Einkauf

Der Einkauf hat in 2007 einen Generationswechsel erfolgreich bewältigt. Nachdem Ende 2006 die bisherigen Mitarbeiterinnen und Mitarbeiter in Altersteilzeit gegangen waren, konnte ein junges Team gefunden werden, das mittlerweile eine hohe Akzeptanz im Haus gewonnen hat. Um den besonderen Bestellanforderungen der Forschungsabteilungen zu entsprechen, wurde ein forschungsspezifisches EDV-Bestellprogramm entwickelt, das Anfang 2008 in den Probebetrieb genommen wird.

Ein weiterer Schwerpunkt lag in der intensiven und systematisierenden Überarbeitung der Wartungsverträge und der Überprüfung der Energieversorgungsverträge.

Technik

Während die Integration des Ersatz- und Erweiterungsbaus II in die bestehende technische Infrastruktur in 2007 abgeschlossen werden konnte, hat die Technik parallel die Sanierung des Seuchenlaborgebäudes vorbereitet. Die planerischen Aktivitäten und die Ausschreibungen der verschiedenen Gewerke wurden von der Technik maßgeblich betreut, sodass im März 2008 die ausführenden Bauaktivitäten starten können.

Systemadministration

Die laufende EDV-Betreuung der kaufmännischen Abteilung ist in 2007 mit einem hohen Servicegrad weitergeführt worden. Darüber hinaus hat der Systemadministrator maßgeblich die Projekte zur Optimierung der Verwaltungsprozesse mitgestaltet und die Zusammenarbeit mit dem EDV-Verantwortlichen der wissenschaftlichen Abteilungen und Forschungsgruppen intensiviert.

Leitung

Die Stellung der kaufmännischen Abteilung als Serviceeinheit hat sich in 2007 weiter stabilisiert. Das Serviceniveau ihrer Dienstleistungen findet eine breite Akzeptanz. Der Prozess ihrer Umgestaltung ist abgeschlossen.

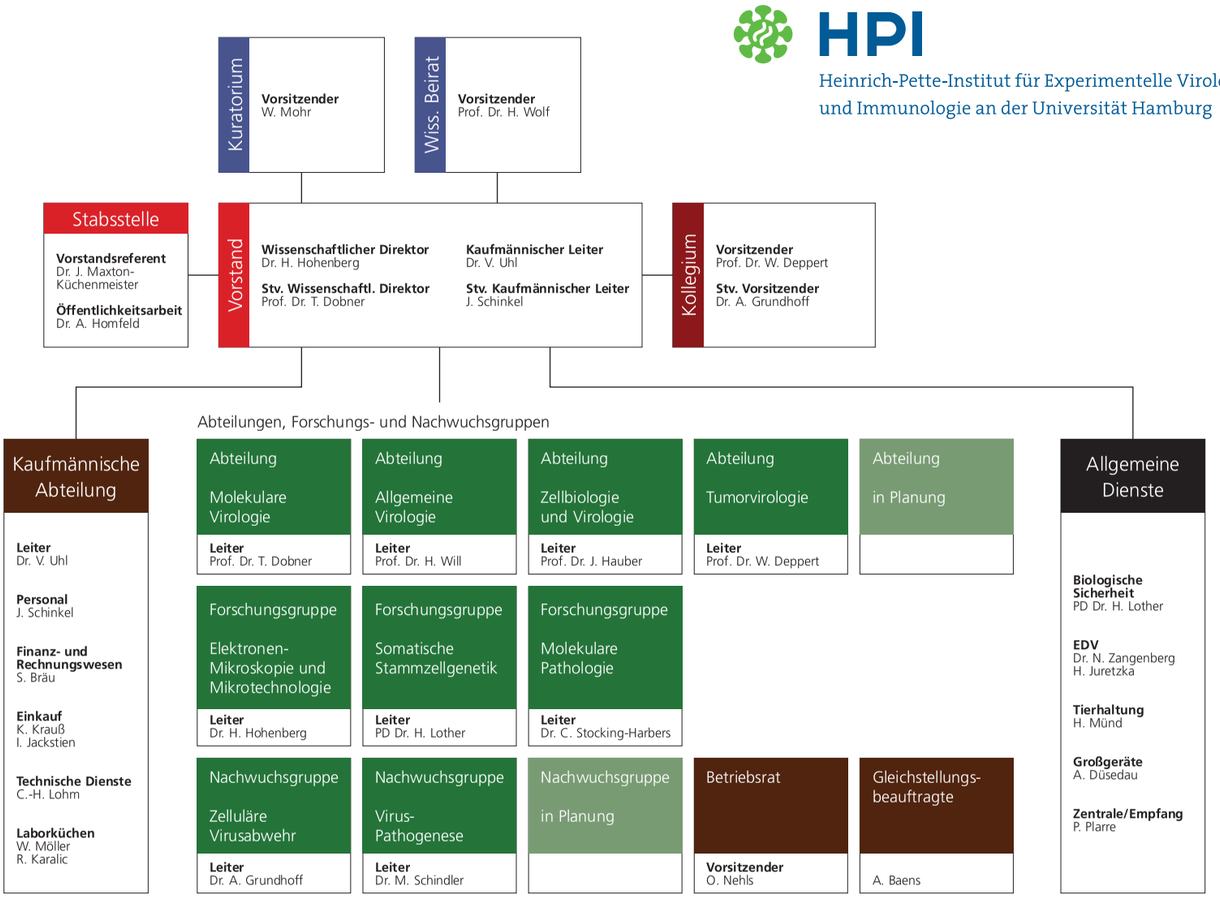
Institutionelle Förderung und Drittmittel 2007 (vorläufiger Endstand 03. 01. 2008)

	Einnahmen	**Ausgaben** Personalkosten	Sachkosten	Investitionen	**Gesamtwert**
Institutionelle Förderung	8.182.886 €	4.420.941 €	2.544.645 €	1.850.227 €	**8.815.813 €**
Zweckgebundene Drittmittel	2.120.488 €	1.186.616 €	540.939 €		**1.727.555 €**
Sonstige Drittmittel	148.101 €	221.372 €	103.545 €		**324.918 €**
Summe Drittmittel	2.268.589 €	1.407.989 €	644.484 €		**2.052.473 €**
Gesamtes HPI	**10.451.476 €**	**5.828.929 €**	**3.189.129 €**	**1.850.227 €**	**10.868.285 €**
davon:					
Grundfinanzierung	7.722.886 €	1.100.569 €	462.601 €		**1.563.170 €**
Gebäude und Technik	460.000 €	247.301 €	929.220 €	1.197.854 €	**2.374.375 €**
Bereich Wissenschaft		3.073.071 €	1.152.824 €	652.373 €	**4.878.267 €**
Summe Institutionelle Förderung	8.182.886 €	4.420.941 €	2.544.645 €	1.850.227 €	**8.815.813 €**
Drittmittel Krebshilfe	644.868 €	484.161 €	181.306 €		**665.468 €**
Drittmittel DFG	567.833 €	430.406 €	132.865 €		**563.271 €**
Drittmittel Bund & Land	430.182 €	47.584 €	8.156 €		**55.740 €**
Drittmittel Stiftungen	328.915 €	214.267 €	105.123 €		**319.389 €**
Drittmittel EU	148.101 €	221.372 €	87.452 €		**308.825 €**
Drittmittel International	6.577 €		6.555 €		**6.555 €**
Drittmittel Unternehmen			16.093 €		**16.093 €**
Drittmittel Diverse	142.114 €	10.198 €	106.934 €		**117.132 €**
Summe Drittmittel	2.268.589 €	1.407.989 €	644.484 €		**2.052.473 €**

Personal der wissenschaftlichen Abteilungen/Gruppen in 2007

Abteilung/ Arb.-Gruppe	Anzahl der Personen			Allgemeine Virologie	Tumor-virologie	Zellbiologie und Virologie	Molekulare Virologie	Elektronen Mikroskopie	Molekulare Pathologie	BBS + FHG-Gastabt.	Zelluläre Virusabwehr	Virus-Pathogenese	Wissensch. Dienste	
Stellenart	Plan	Dritt	Gast											
Wiss. Abteilungsleiter														
Planstellen-Personal	4			1	1	1	1	2	1	1	1	1	1	4
Wiss. Angstellte														
Planstellen-Personal	19			3	3	3	3		2	1	2			8
Drittmittel-Personal		10		4	3				1	1	2			4
Gäste			16	2	6	1	1			1		1		4
Doktoranden (IIA-halbe)														
Planstellen-Personal	4								6					22
Drittmittel-Personal		22		2	9	3		1	1	1		1	1	4
Gäste			5	2		2			2					
Diplomanden														
Planstellen-Personal	0													
Drittmittel-Personal		0							2		1			2
Gäste			20	4	6	2	3							
Studenten														
Planstellen-Personal	1													
Drittmittel-Personal		1				1		2						8
Gäste			15	2		1	10							
Ingenieure														
Planstellen-Personal	3													
Drittmittel-Personal		0												
Gäste			0											
Techn. Assistenten														
Planstellen-Personal	14			2	3	3	1	1		1		1		4
Drittmittel-Personal		9		0	5	2								
Gäste			9	3	3	2								
Fremdsprach. Ang.														
Planstellen-Personal	4			1	1	1	1		2					2
Drittmittel-Personal		0												
Gäste			0											
Helfer in den Spülkuchen														
Planstellen-Personal	8			2	2	2								
Drittmittel-Personal		0												
Gäste			0											
zusammen				28	42	24	20	8	22	4	8	4	4	

HPI
Heinrich-Pette-Institut für Experimentelle Virologie und Immunologie an der Universität Hamburg



Organigramm, Stand: Dezember 2007