Leibniz Institute
for Natural Product Research and Infection Biology
Hans Knöll Institute

# Research Report 2006/2007

# HKI Research Report 2006/2007

The Leibniz Institute for Natural Product Research and Infection Biology – Hans Knöll Institute (HKI) – has continued to develop considerably in the past two calendar years. The greatest challenge during these years was the institute's evaluation in June 2007. All employees were extremely curious to learn how the partial new focussing of the institute would be assessed by competent external scientists. Even though there has been no official statement at the time of the writing of this report, all signals seem to indicate a very positive result which will support us on our way and will encourage us in taking new steps.

The consequent development of the Hans Knöll Institute was also accelerated by the successful establishment of new structural units. This includes the foundation of the department "Microbial Pathogenicity Mechanisms" which was taken over by Dr. Bernhard Hube of the Robert Koch Institute Berlin who at the same time accepted a chair for "Microbial Pathogenicity" at the Faculty of Biology and Pharmaceutics at the Friedrich Schiller University of Jena. Thus, the institute was able to attract an internationally renowned scientist who will essentially stimulate research with the human-pathogenic yeast *Candida albicans* at the HKI. And also the posts of two junior group leaders could be filled. The junior group "Microbial Biochemistry and Physiology" will be headed by Dr. Matthias Brock, and the junior group "Cellular Immunobiology" will be managed by Dr. Mihály Józsi.
On initiative of the HKI, the first international Leibniz Graduate School "International Leibniz Research School for Microbial and Biomolecu-

## INTRODUCTION | VORWORT

Das Leibniz-Institut für Naturstoff-Forschung und Infektionsbiologie – Hans-Knöll-Institut – hat sich auch in den letzten beiden Kalenderjahren wesentlich weiter entwickelt. Die größte Herausforderung dieser Jahre war die Evaluierung des Instituts im Juni 2007. Alle Mitarbeiter waren sehr gespannt, wie die partielle Neuausrichtung des Instituts von kompetenten Wissenschaftlern von außen beurteilt werden würde. Auch wenn es beim Abfassen dieses Berichts noch keine offizielle Stellungnahme gab, deuteten doch alle Signale auf ein sehr positives Votum hin, was uns in unserem Weg bestärkt und in unseren weiteren Schritten ermutigt.

Die konsequente Entwicklung des Hans-Knöll-Instituts wurde auch durch die erfolgreiche Etablierung neuer Struktureinheiten beschleunigt. Dazu zählt die Gründung der Abteilung „Mikrobielle Pathogenitätsmechanismen", deren Leitung Herr Dr. Bernhard Hube vom Robert-Koch-Institut in Berlin übernommen hat und der gleichzeitig auf einen Lehrstuhl für „Mikrobielle Pathogenität" an der Biologisch-Pharmazeutischen Fakultät der Friedrich-Schiller-Universität Jena berufen wurde. Dadurch ist es dem Institut gelungen, einen international renommierten Wissenschaftler zu gewinnen, der die Forschung an der human-pathogenen Hefe *Candida albicans* im HKI maßgeblich befruchtet. Auch die Positionen zweier Nachwuchsgruppen konnten besetzt werden. Die Nachwuchsgruppe „Mikrobielle Biochemie und Physiologie" wird von Dr. Matthias Brock und die Nachwuchsgruppe „Zelluläre Immunbiologie" von Dr. Mihály Józsi geleitet.
Auf Initiative des HKI wurde die erste internationale Leibniz-Graduiertenschule „International Leibniz Research School for Microbial and Biomolecular Interactions" gegründet,

lar Interactions" was founded which already in spring 2006 was able to welcome the first PhD students which were selected in a competitive procedure. This graduate school comprises scientists of the Hans Knöll Institute, of the Friedrich Schiller University as well as of the Max Planck Institute for Chemical Ecology in Jena.
Beside the evaluation, the Excellence Competition of the federal government and the single federal states, organized by the Deutsche Forschungsgemeinschaft (DFG: German Research Association) was another milestone in the year 2007. The graduate school "Jena School for Microbial Communication", coordinated by Prof. Erika Kothe, Prof. Wilhelm Boland and Prof. Axel Brakhage was successfully presented to the DFG in Bonn in June 2007, and was recommended for promotion in October 2007. The said school is the only excellence project of the Free State of Thuringia that was selected for promotion in the framework of this highly competitive procedure. The HKI contributed fundamentally to this development.

The year 2007 was also characterized by the intensive development of an application for a Centre for Innovation Competence "ZIK Septomics" which was prepared together with Prof. Konrad Reinhart (Clinic for Anaesthesiology and Intensive Therapy, University Hospital Jena) and Prof. Eberhard Straube (Institute for Medical Microbiology, University Hospital Jena), and which already managed to achieve the second stage. This centre is intended to link basic research and clinical research in Jena in the field of sepsis. It is a joint application of the Friedrich Schiller University, the University Hospital and the Hans Knöll Institute.

die bereits im Frühjahr 2006 die ersten, in einem kompetitiven Verfahren ausgewählten Doktoranden begrüßen konnte. An dieser Graduiertenschule beteiligen sich Wissenschaftler des Hans-Knöll-Instituts, der Friedrich-Schiller-Universität und des Max-Planck-Instituts für chemische Ökologie aus Jena.
Neben der Evaluierung war der Exzellenzwettbewerb des Bundes und der Länder durch die Deutsche Forschungsgemeinschaft ein weiterer außerordentlicher Meilenstein des Jahres 2007. Die von Prof. Erika Kothe, Prof. Wilhelm Boland und Prof. Axel Brakhage koordinierte Graduiertenschule „Jena School for Microbial Communication" wurde im Juni 2007 erfolgreich in Bonn bei der DFG vorgestellt und im Oktober 2007 zur Förderung empfohlen. Es handelt sich um das einzige Exzellenzprojekt aus dem Freistaat Thüringen, welches im Rahmen des hochkompetitiven Verfahrens gefördert wird. Das HKI hat dazu einen wesentlichen Beitrag geleistet.

Das Jahr 2007 war auch durch die intensive Entwicklung eines Antrags für ein Zentrum für Innovationskompetenz „ZIK Septomics", der zusammen mit Prof. Konrad Reinhart (Klinik für Anästhesiologie und Intensivtherapie, Universitätsklinikum Jena) und Prof. Eberhard Straube (Institut für Medizinische Mikrobiologie, Universitätsklinikum Jena) ausgearbeitet wurde und bereits erfolgreich die zweite Runde erreicht hat. Dieses Zentrum soll im Bereich Sepsis die Grundlagenforschung und die klinische Forschung in Jena verknüpfen. Es handelt sich um einen gemeinsamen Antrag der Friedrich-Schiller-Universität, des Universitätsklinikums und des Hans-Knöll-Instituts.
Einen weiteren Schwerpunkt im Jahre 2007 bildete die Arbeit an dem im Rahmen des Paktes für Forschung und Innovation bewil-

Another focus of the year 2007 was the work on the research project "Functional Genome Analysis of the Dermatophyte *Arthroderma benhamiae*" which was approved in the framework of the Pact for Research and Innovation. Thanks to this project a complete genome of an inferior eukaryote will be sequenced in Germany, in particular in Jena, for the first time – which is important for human beings under infection-related aspects.

In this connection, the HKI was able to successfully establish the prototype of a PET/CT which can be used for analyses of highly complex infections and their therapy with living animals. It is to contribute to work on questions which so far could not be investigated or addressed, respectively.

We are very happy and proud that employees of the Hans Knöll Institute were awarded competitive posts. PD Dr. Jürgen Wendland accepted a chair for yeast biology at the Carlsberg Laboratory in Copenhagen/Denmark. Dr. Imke Schmidt became an assistant professor at the University of Minnesota.

Furthermore, other projects enlarge the already existing research works of the Hans Knöll Institute, such as "ERA-NET PathoGenoMics", which does research in the field of pathogenicity mechanisms with *Candida glabrata*, or the association Zoonoses in which the virulence of zoonotic Chlamydia is being investigated. In addition, the Hans Knöll Institute participates in the EU programme "Management Strategies for Invasive Aspergillosis" (MANASP) which is to develop new treatment strategies against invasive aspergilloses, as well as in another EU supported project in which new medication against tuberculosis shall be developed.

ligten Forschungsprojekt „Funktionelle Genomanalyse des Dermatophyten *Arthroderma benhamiae*". Dank dieses Projekts wird erstmals ein komplettes Genom eines niederen Eukaryonten – welcher unter infektiologischen Aspekten für den Menschen wichtig ist – in Deutschland, insbesondere in Jena, sequenziert.

In diesem Zusammenhang konnte das HKI auch erfolgreich den Prototyp eines PET/CT etablieren, das für die Analyse von hochkomplexen Infektionen und deren Therapie im lebenden Tier eingesetzt werden kann. Es soll dazu beitragen, bisher nicht untersuchbare oder nicht adressierbare Fragestellungen zu bearbeiten.

Wir freuen uns sehr, dass Mitarbeiter des Hans-Knöll-Institutes kompetitiv vergebene Positionen erhielten. PD Dr. Jürgen Wendland hat eine Professur für Hefebiologie am Carlsberg Laboratory in Kopenhagen, Dänemark angenommen. Dr. Imke Schmidt wurde Assistant Professor an der University of Minnesota.

Desweiteren bereichern andere Projekte die schon vorhandenen Forschungsarbeiten des Hans-Knöll-Instituts, wie das „ERA-NET PathoGenoMics", das die Pathogenitätsmechanismen bei *Candida glabrata* erforscht oder der Verbund Zoonosen, in dem die Virulenz zoonotischer Chlamydien untersucht wird. Zusätzlich beteiligt sich das Hans-Knöll-Institut an dem EU-Programm „Management strategies for invasive aspergillosis" (MANASP), in dem neue Behandlungsstrategien gegen invasive Aspergillosen entwickelt werden sollen sowie an einem weiteren von der EU geförderten Projekt, in dem neue Medikamente gegen Tuberkulose entwickelt werden.

Die hohe Qualität der Forschung des Hans-Knöll-Instituts äußert sich auch in Publikationen in den renommiertesten Zeitschriften, wie „Nature", „Nature Chemical Biology",

The high quality of research at the Hans Knöll Institute is also displayed in publications in the most renowned journals, such as “Nature”, “Nature Chemical Biology”, “PLoS Pathogens”, “EMBO Journal”, “Current Biology” or “Applied Chemistry International Edition”, just to name a few of them.
There is a close connection between this high quality research and the positive number of awards and prizes which were won by employees of the Hans Knöll Institute in the reporting period: e. g. Dr. Andrea Walther was awarded the 2006 Junior Award of the Leibniz Association for her excellent work in the laboratory of PD Dr. Jürgen Wendland at the HKI. Stefan Heinen won the 2006 Hans Hench Award for Clinical Immunology of the German Association for Immunology, and Prof. Axel Brakhage was awarded the Heinz P. R. Seeliger Award for Medical Bacteriology or Mycology. The head of the Department of Infection Biology, Prof. Peter Zipfel, was awarded the 2007 Heinz Spitzbart Prize of the European Association for Infectious Diseases in Obstetrics and Gynaecology. Dr. Antje Albrecht, Judith Behnsen and Dr. Claudio Kupfahl each were given the publication awards of the German-speaking Mycological Association, and Betty Wächtler received the Hans Rieth Poster Award of the same association. We are in particular happy about the awarding of the Order of Merit of the Federal Republic of Germany to our long-standing member of the Board of Trustees, Mr. Wilfried Mohr of Hamburg.
The activities of the Hans Knöll Institute also find their expression in the organization of numerous meetings and conferences. This includes joint meetings of the research area

„PLoS Pathogens“, „EMBO Journal“, „Current Biology“ oder „Angewandte Chemie International Edition“, um nur einige zu nennen.

Zusammenhängend mit dieser qualitativ hochwertigen Forschung ist die erfreuliche Zahl von Preisen, die im Berichtszeitraum von Mitarbeitern des Hans-Knöll-Instituts gewonnen wurden: So wurde Dr. Andrea Walther mit dem Nachwuchspreis der Leibniz-Gemeinschaft 2006 für ihre hervorragenden Arbeiten im Labor von PD Dr. Jürgen Wendland im HKI ausgezeichnet. Herr Stefan Heinen gewann den Hans-Hench-Preis für Klinische Immunologie der Deutschen Gesellschaft für Immunologie 2006 und Prof. Axel Brakhage wurde mit dem Heinz P. R. Seeliger-Preis für Medizinische Bakteriologie oder Mykologie geehrt. Der Leiter der Abteilung Infektionsbiologie, Prof. Peter Zipfel, erhielt den Heinz-Spitzbart-Preis 2007 der Europäischen Gesellschaft für Infektionskrankheiten in der Geburtshilfe und Gynäkologie. Dr. Antje Albrecht, Judith Behnsen und Dr. Claudio Kupfahl wurden jeweils mit Publikationspreisen der Deutschsprachigen Mykologischen Gesellschaft geehrt und Betty Wächtler erhielt den Hans-Rieth-Posterpreis der selben Gesellschaft. Besonders gefreut hat uns die Verleihung des Bundesverdienstkreuzes am Bande an unser langjähriges Kuratoriumsmitglied, Herrn Wilfried Mohr aus Hamburg.

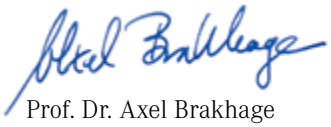
Die Aktivitäten des Hans-Knöll-Instituts äußern sich nicht zuletzt auch in der Ausrichtung einer großen Zahl von Tagungen. Dazu gehören gemeinsame Tagungen des Bereiches Bioinformatik des Hans-Knöll-Instituts mit dem Jenaer Centrum für Bioinformatik und der Deutschen Forschungsgemeinschaft, die von PD Dr. Reinhard Guthke und Dr. Michael Pfaff organisiert wurden sowie Symposien

Bioinformatics of the Hans Knöll Institute and the Jena Centre for Bioinformatics with the Deutsche Forschungsgemeinschaft which were organized by PD Dr. Reinhard Guthke and Dr. Michael Pfaff, as well as conferences of the graduate school "International Leibniz Research School", and two workshops with the title "Theragnostic Workshop on Severe Infections Leading to Sepsis" organized by Prof. Dr. Axel Brakhage in collaboration with Prof. Konrad Reinhart and Prof. Eberhard Straube, and also colloquia in the framework of DFG Priority Programmes, e. g. the DFG Priority Programme 1160 "Colonisation and Infection by human-pathogenic fungi" which was organized by Prof. Axel Brakhage and Dr. Olaf Kniemeyer as well as colloquia in the framework of EU programmes, such as the international "Proteomics Workshop".

I would like to thank all of my colleagues at the HKI. Only on the basis of intensive team work it is possible to maintain and increase high quality scientific performance. I would also like to thank all employees for collaborating with the writing of this 2006/2007 Report, in particular Dr. Michael Ramm.

The gentle reader I wish joy when reading this report.

Jena, June 2008
Prof. Dr. Axel Brakhage
Director of the HKI



Prof. Dr. Axel Brakhage

der Graduiertenschule „International Leibniz Research School", zwei Workshops mit dem Titel „Theragnostic Workshop on Severe Infections Leading to Sepsis" organisiert von Prof. Axel Brakhage in Kollaboration mit Prof. Konrad Reinhart und Prof. Eberhard Straube und auch Kolloquien im Rahmen von DFG-Schwerpunktprogrammen, z. B. des DFG-Schwerpunktprogrammes 1160 „Kolonisation und Infektion durch human-pathogene Pilze", das von Prof. Axel Brakhage und Dr. Olaf Kniemeyer organisiert wurde sowie auch Kolloquien im Rahmen von EU-Programmen, wie etwa der internationale „Proteomics Workshop".

Für die ausgezeichnete Kooperation möchte ich mich bei allen Kolleginnen und Kollegen des HKI bedanken. Nur durch ausgesprochene Teamarbeit ist es möglich, die hohe qualitative wissenschaftliche Leistung des HKI zu halten und weiter zu steigern. Für die Mitarbeit an diesem Report 2006/2007 danke ich allen Mitarbeitern des HKIs, insbesondere aber Dr. Michael Ramm.

Dem geneigten Leser wünsche ich viel Freude beim Lesen dieses Reports.

Jena, im Juni 2008
Prof. Dr. Axel A. Brakhage
Direktor des HKI

Axel A. Brakhage

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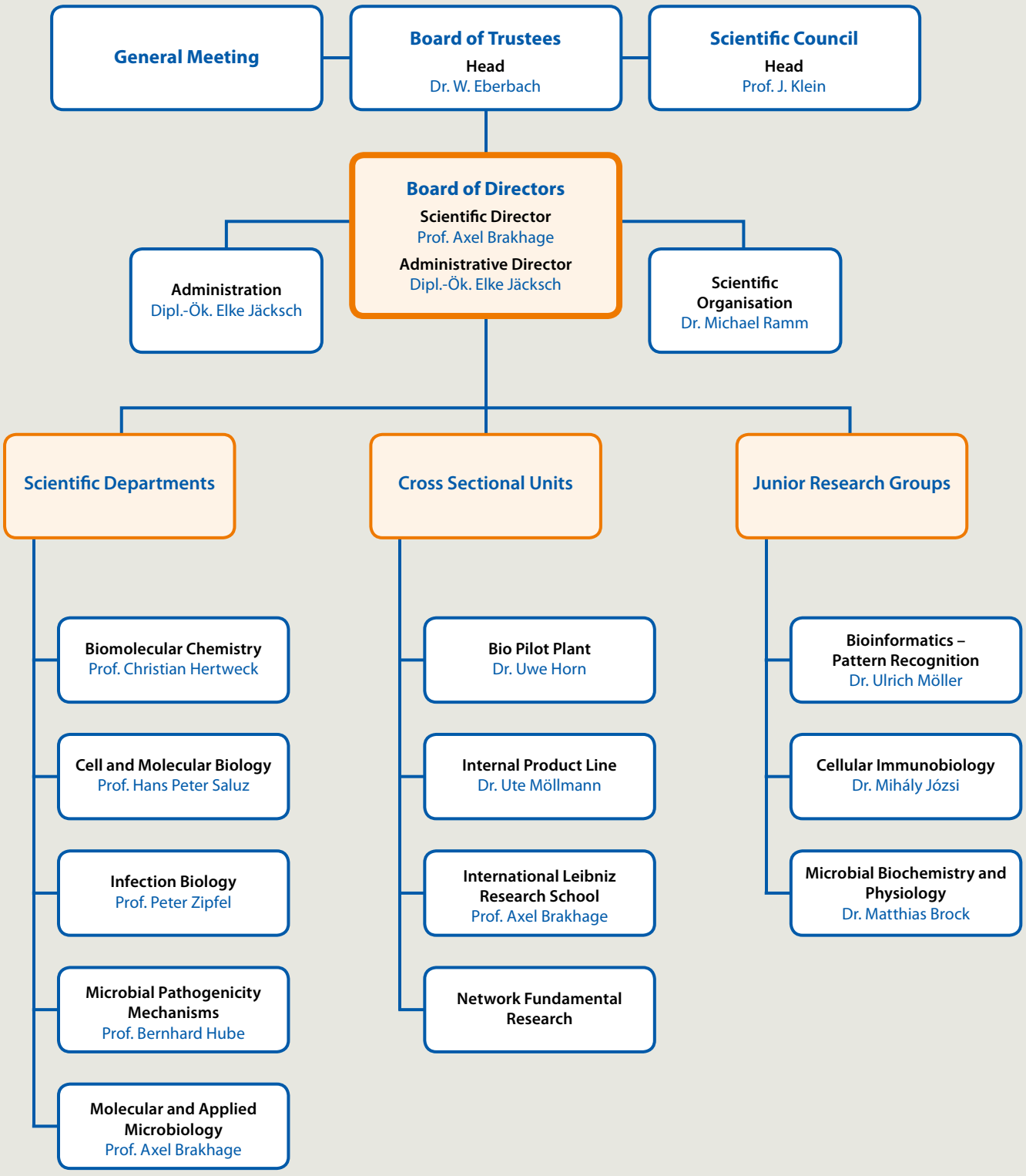
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# Department of Biomolecular Chemistry

# Department of Biomolecular Chemistry

Research in the Department of Biomolecular Chemistry, headed by Prof. Christian Hertweck, focuses on various aspects of microbial secondary metabolism. The group covers the chemical and biochemical expertise in modern natural product research (structure elucidation, biosynthesis, enzymology, synthesis, interactions through biomolecules). In addition to the basic research projects, the department operates the institute's state-of-the-art analytical facilities (NMR, MS-techniques, MALDI-TOF) and supports other HKI departments (in particular MAM, IB) with metabolome and proteome analyses. In the course of Prof. Hertweck's appointment in early 2006 the instrumental set-up was complemented with new nanoflow- and chip-LC/MS machines for microanalyses. In addition, highly sensitive analytical methods for the detection of metabolites and proteins were optimized.

The department also harbors the natural product discovery group (headed by Dr. Isabel Sattler), which is specialized in the isolation and structural elucidation of natural products by bioactivity-guided screening. This work is funded by DBU and BMBF and supported by various collaborations with industrial partners. Furthermore, the research group maintains the institute's central compound library. For the discovery of new potential therapeutics we investigate primarily novel types of

## INTRODUCTION | EINLEITUNG

**Head**
**Prof. Dr. Christian Hertweck**

Die Abteilung Biomolekulare Chemie befasst sich unter der Leitung von Prof. Christian Hertweck mit der Chemie und Biologie des facettenreichen Sekundärmetabolismus von Bakterien und Pilzen. In der Abteilung sind die chemischen und biochemischen Arbeitsgebiete der modernen Naturstoff-Forschung integriert (Strukturaufklärung, Biosynthese, Enzymmechanismen, Synthese und Naturstoff-basierte Interaktionen).

Die für das Institut essentielle Hochleistungsanalytik (NMR, MS, MALDI-TOF) wird in der Abteilung Biomolekulare Chemie betrieben. Sie verfügt über ein breites Methodenspektrum zur Strukturaufklärung von niedermolekularen Verbindungen und zur Proteomanalyse, durch die insbesondere die Abteilungen Molekulare und Angewandte Mikrobiologie und Infektionsbiologie unterstützt werden. Mit der Berufung von Prof. Christian Hertweck zum 01.02.2006 wurde die instrumentelle Ausstattung durch neue Nanoflow- und Chip-LC/MS – Geräte zur Mikroanalytik ergänzt. Zudem wurden neue, hochempfindliche Verfahren zur Analytik niedermolekularer Verbindungen und zur Proteinanalytik optimiert.

In der Abteilung Biomolekulare Chemie ist die Forschungsgruppe Naturstoff-Screening um Frau Dr. Isabel Sattler verankert, die sich auf die Isolierung und Strukturaufklärung von Naturstoffen über Aktivitäts-basiertes Screening spezialisiert hat. Diese Arbeiten werden DBU- und BMBF-gefördert und in zahlreichen Industriekooperationen durchgeführt. Zudem führt die Forschungsgruppe die zentrale Substanzbibliothek des Instituts.

microorganisms (endophytes, symbionts, little explored microorganisms) and apply *genome*-mining strategies (activation of silent gene clusters).

A major research area of the department is the investigation of biosynthetic pathways by a combination of chemical and biological methods. In nature, structural and functional diversity is governed by multienzyme processing lines that lead to highly complex compounds. To gain insights into the mechanisms and the evolution of the synthetic machineries the group investigates the functions of biosynthesis gene clusters and mechanisms selected intriguing enzymes. For this purpose, we also do structural studies in collaboration. The majority of our projects is conducted in national and international scientific networks (EU, BMBF "GenoMik", DFG SPP1152). In addition to understanding natural evolution of metabolic diversity we aim at harnessing the biosynthetic potential to produce novel natural product derivatives. In particular, biosynthetic pathways of pharmacologically relevant polyketides are investigated. Modern organic synthetic methods complement the microbes' biosynthetic capabilities. The targeted chemical derivatization of therapeutics is in part supported by an NIH-funded collaborative work.

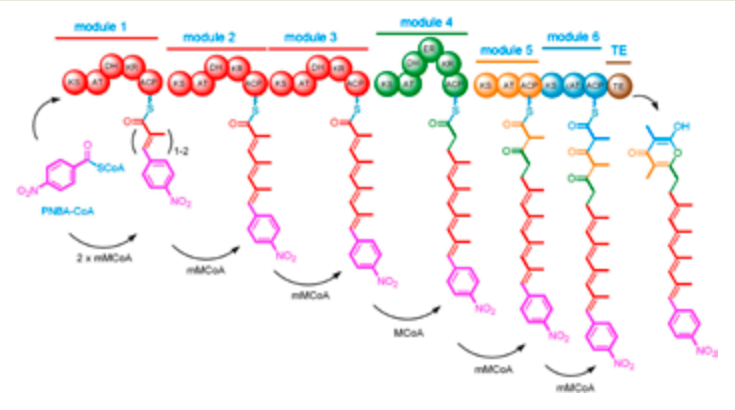
Natural products represent mediators of bio-

Zur Suche nach neuen Naturstoffen werden vorwiegend neue Organismentypen (Endophythen, Symbionten, wenig untersuchte Mikroorganismen) verwendet und Genome Mining-Strategien eingesetzt. Ein Schwerpunkt der Abteilung Biomolekulare Chemie liegt in der Aufklärung von mikrobiellen Biosynthesewegen über biologische und chemische Methoden. Die strukturelle und funktionelle Diversität entsteht in der Natur über Multi-Enzym-Prozesslinien, die zu hochkomplexen Verbindungen führen. Um die Mechanismen und die Evolution der Synthesemaschinerien biologischer Systeme zu verstehen, studiert die Arbeitsgruppe die Biosynthese-Gencluster und ausgewählte Enzyme. In Kollaboration werden hierzu auch strukturbiologische Projekte durchgeführt. Die meisten dieser interdisziplinären Arbeiten werden in überregionalen Netzwerken (EU, BMBF „GenoMik", DFG SPP1152) bearbeitet. Die Projekte haben nicht nur zum Ziel, ein besseres Verständnis von der Evolution metabolischer Diversität zu bekommen, sondern auch das natürliche Biosynthesepotential zu nutzen, um neue Naturstoff-Derivate darzustellen. Bei den hierzu angewandten biokombinatorischen Techniken stehen Biosynthesewege pharmakologisch relevanter Polyketide im Mittelpunkt. Moderne Synthesemethoden und Biotransformationen komplementieren die Biosyntheseleistung der Mikroorganismen. Die gezielte chemische Derivatisierung von Wirkstoffen wird auch in Kooperation im Rahmen eines NIH-Projektes durchgeführt.

Naturstoffe sind Mediatoren der biologischen Kommunikation, die im Laufe der Evolution

logical communication, and their specific functions have developed during evolution. They play a key role in the interaction of microorganisms, in symbiosis and also in pathogenesis, e.g. in fungal infections. We have discovered the first case, in which it is not the fungus that produces a mycotoxin, but bacterial symbionts that live within the fungal mycelium. This unique alliance of bacteria (*Burkholderia sp.*) and fungi (*Rhizopus microsporus*) produces the antimitotic polyketide rhizoxin, the causative agent of rice seedling blight. In the department various aspects of this tripartite microbial interaction are being investigated, such as evolution of symbiosis and molecular basis of toxin biosynthesis. The biomolecular interactions in fungal-bacterial associations are jointly investigated with research groups at the HKI, the FSU, and the Max-Planck-Institute for chemical Ecology in the context of the graduate schools ILRS and JSMC.

auf biologische Aktivität selektioniert worden sind. Sie spielen in der Interaktion von Mikroorganismen, bei Symbiosen und auch als Virulenzdeterminanten, zum Beispiel bei Pilzinfektionen, eine wichtige Rolle. Wir haben das erste Beispiel beschrieben, in dem nicht der Pilz ein Mykotoxin bildet, sondern bakterielle Symbionten, die im Pilzmyzel leben (Partida-Martinez and Hertweck, 2005). Die einzigartige Allianz aus Bakterien (*Burkholderia sp.*) und Pilzen (*Rhizopus microsporus*) bildet das antimitotisch wirksame Rhizoxin, das eine Schlüsselrolle bei der Reiskeimlingsfäule spielt. In der Abteilung Biomolekulare Chemie werden zahlreiche Aspekte dieser mikrobiellen Interaktion studiert, wie die Evolution der Symbiose und die molekulare Grundlage der Toxin-Biosynthese. Die biomolekularen Interaktionen in Pilz-Bakterien-Symbiosen sind Themen stark vernetzter Gemeinschaftprojekte der Arbeitsgruppen am HKI, der FSU und des Max-Planck-Instituts für chemische Ökologie im Rahmen der Graduiertenschulen ILRS und JSMC.



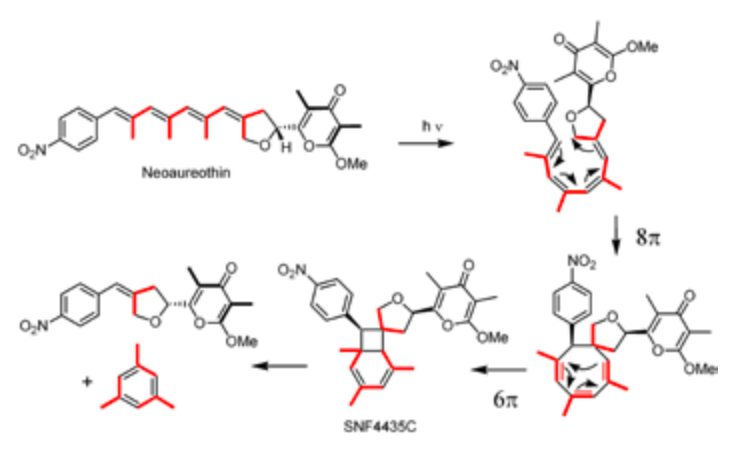




**Figure 1-1**
Biosynthetic processing line leading to the antibiotic neoaureothin; formation of orinocin from neoaureothin via photo-induced "polyene splicing"

## 1 Biosynthetic Mechanisms and Processing Lines

Group Leader: Christian Hertweck

Polyketides constitute a structurally highly diverse group of natural products that possess broad ranges of pharmacological properties and represent a major source for novel therapeutics. The diversity of polyketide metabolites is a result of a number of programmed events governed by polyketide synthases, which assemble highly complex molecules from simple fatty acid building blocks. Studying the molecular basis of polyketide biosynthesis by chemical and biological methods may set the basis for improving the production and for rationally engineering novel derivatives with altered bioactivity profiles.

Bacterial type I polyketide synthases represent molecular processing lines that are usually colinear with the structure of the metabolite. This principle of colinearity has served for re-designing polyketide pathways and to rationalize the evolution of the thiotemplate systems by gene duplication, deletion, as well as loss and gain of function. We have cloned and sequenced several gene loci encoding the biosynthesis of bacterial polyketides. Analyses of the aureothin and neoaureothin pathways in *Streptomyces* spp. revealed that PKSs are rare exceptions to the rule of colinearity since individual modules catalyze two rounds of chain elongations. On the basis of phylogenetic studies we could deduce that the aureothin pathway has most likely emerged from neoaureothin biosynthesis through gene deletion. Furthermore, the phylogenetic relationships also revealed which nor PKS module acts in an iterative fashion. More recently, we isolated the lower homologue orinocin as a new member of the aureothin family. We were surprised to find that orinocin is not the product of a truncated PKS processing line, but is derived from neoaureothin by an unprecedented photoinduced rearrangement cascade that is terminated by a retro-[2+2]-cycloaddition. We

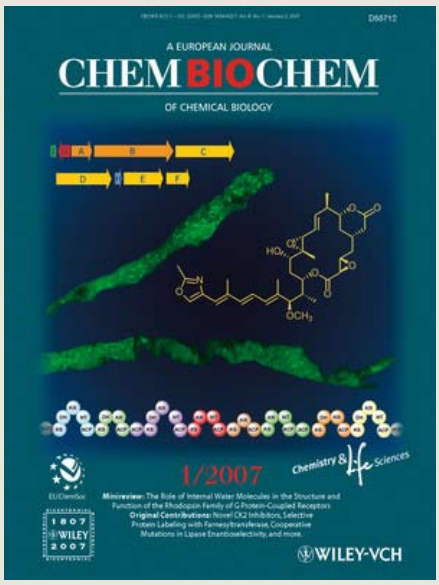



**Figure 1-2**
ChemBioChem Cover showing rhizoxin-producing endofungal bacteria *(Burkholderia rhizoxinica)*, the rhizoxin biosynthesis gene cluster and a fragment of the PKS processing line.

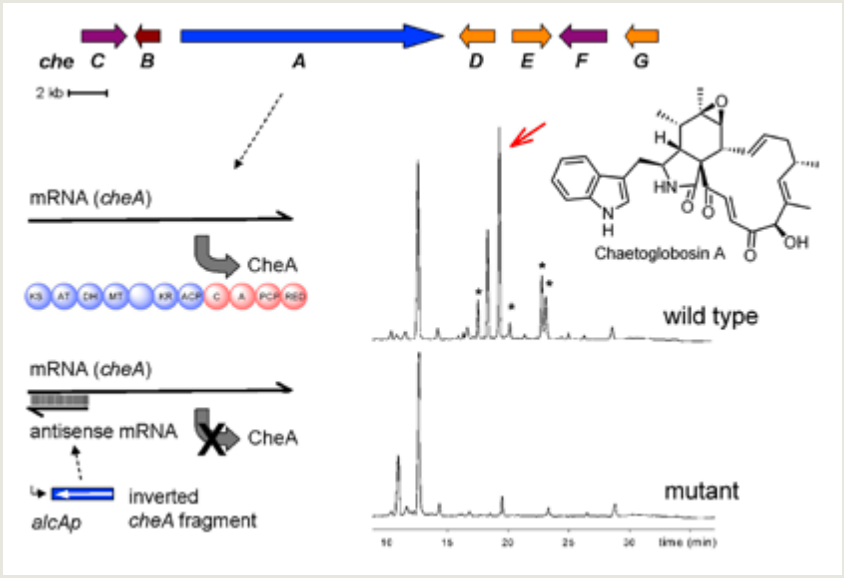



**Figure 1-3**
Organization of the cytochalasin (chaetoglobosin) biosynthesis gene cluster from *Penicillium expansum* and its functional evidence by RNA silencing.

termed this novel process resulting in the excision of an aromatic side product (here: mesitylene) "polyene splicing" (collaboration Prof. Trauner, UC Berkeley).

Rhizoxin is a potent antimitotic macrolide that very efficiently binds to β-tubulin and due to its excellent *in vitro* antitumoral activities it has undergone extensive clinical trials as a potential anticancer drug candidate. We have located, cloned and sequenced the entire ~81 kb gene locus encoding rhizoxin biosynthesis in the genome of the bacterial endosymbiont of the rice pathogenic fungus *R. microsporus*. Analyses of the deduced *rhi* gene functions provided valuable insights into the giant rhizoxin NRPS-PKS assembly line, which deviates from textbook type I PKS systems in various aspects. Most importantly, the module architecture and the deduced pathway intermediates suggest that the rare β-branching results from an unprecedented conjugate addition of an acyl anion synthon to an intermediary enoyl moiety. This work reports the first example of a biosynthetic gene cluster from endofungal bacteria, and the unprecedented genetic manipulation of a cultured endosymbiont. From a biosynthetic viewpoint the analysis of the *rhi* gene cluster is the starting point for rationally engineering novel antitumoral rhizoxin derivatives.

Cytochalasans comprise a diverse group of structurally intriguing fungal metabolites that are well-known for their specific binding to actin filaments. We have cloned and sequenced the first gene cluster coding for the biosynthesis of a cytochalasan. We have established a specific RNA silencing method for analyzing cytochalasan biosynthesis in *Penicillium*. The successful inactivation provided direct evidence that the rare fungal PKS-NRPS hybrid synthase CheA plays an essential role in chaetoglobosin formation. According to its architecture the multifunctional enzyme catalyzes polyketide assembly as well as the attachment of L-Trp and reductive downloading. The latter

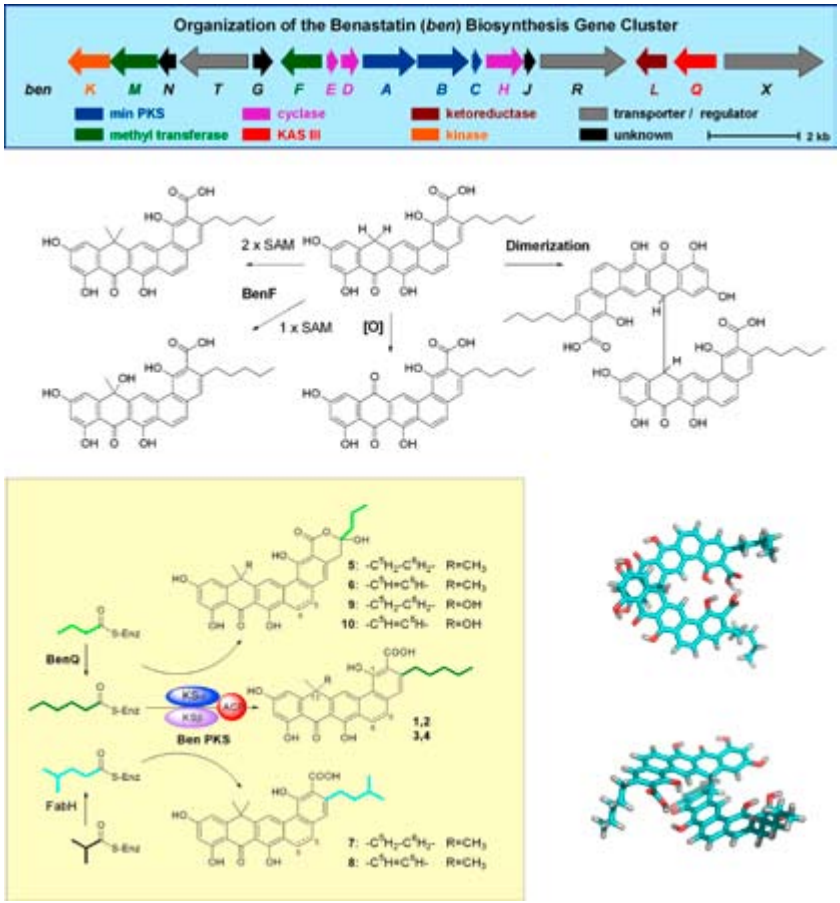



**Figure 2-1**
Organization of the benastatin biosynthesis gene cluster and selected results from pathway engineering.

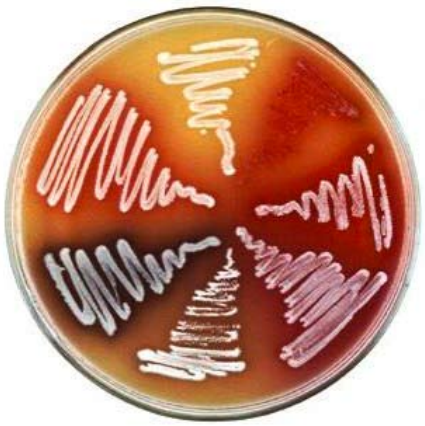


**Figure 2-2**
Agar plate with variants of a polyketide-producing *Streptomyces* strain.

sets the stage for the immediate formation of the 1,5-dihydro-pyrrol-2-one dienophile, which is capable of reacting with the polyene portion in a Diels-Alder fashion. This work allows the first insight into the remarkable cytochalasan pathway and provides the ground for engineering analogs.

## 2 Engineered Biosynthesis of Aromatic Polyketides

Group Leaders: Zhongli Xu, Christian Hertweck

Bacterial aromatic polyketides represent a large group of structurally diverse natural products many of which are medicinally relevant (e.g. tetracyclines, anthracyclines). With the advent of molecular tools and recombinant methods applicable to actinomycetes, it has become feasible to investigate bacterial aromatic polyketide biosynthesis at the genetic and biochemical levels, which has finally set the basis for engineering novel natural product derivatives. We are exploring the biosynthetic potential by rational mutagenesis and gene swapping and have applied this to various antibiotic biosynthetic pathways leading to polyphenols such as resistomycin, chartreusin and benastatin. The benastatins are pentacyclic polyphenols that inhibit glutathione-S-transferases and induce apoptosis. We have recently cloned and sequenced the entire gene locus (*ben*) encoding benastatin biosynthesis. The cluster identity was unequivocally proven by deletion of flanking regions and heterologous expression in *S. albus* and *S. lividans*. Inactivation and complementation experiments revealed that a KSIII component (BenQ) similar to FabH is crucial for providing and selecting the rare hexanoate PKS starter unit. In the absence of BenQ, several novel penta- and hexacyclic benastatin derivatives with antiproliferative activities are formed. The most intriguing observation is that the *ben* PKS can utilize typi-

**Figure 3-1**
General sketch showing functionalized macrolide.

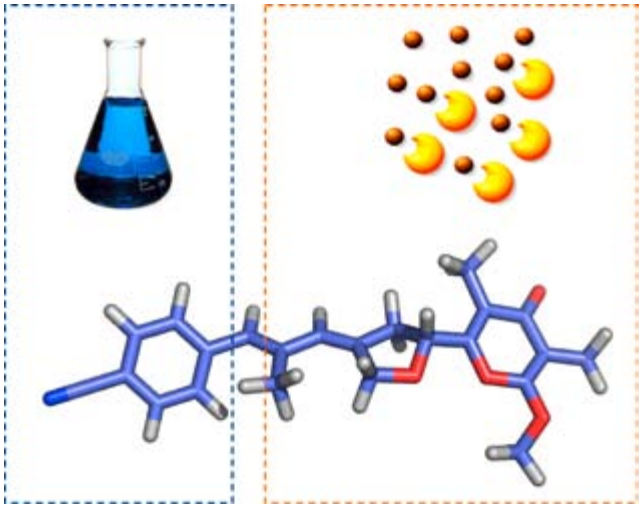


**Figure 3-2**
Merging the synthetic potential of chemistry and biology.

cal straight and branched fatty acid synthase primers. If shorter straight-chain starters are utilized, the length of the polyketide backbone is increased, resulting in the formation of an extended, hexacyclic ring system reminiscent of proposed intermediates in the griseorhodin and fredericamycin pathways. Further gene inactivation experiments yielded several structurally intriguing bis-anthrone derivatives and various other previously unknown benastatin derivatives (Fig. 2).

## 3 Semisynthesis and Chemobiosynthesis of Natural Product Derivatives

Group Leaders: Peter Gebhardt, Christian Hertweck

Natural products are still the most important source for new antibiotics and antitumoral therapeutics. However, these compounds may be sub-optimal in regard of activity, selectivity, availability and unwanted side effects.

We are currently exploring ways to combine biosynthetic and synthetic methods to yield variants of pharmaceutically relevant natural products.

As a complementation of metabolic engineering strategies, the chemical derivatization of natural products can be a powerful approach to generate yet unexplored chemical diversity. Macrolides have emerged as very promising scaffolds for the semi-synthesis of a large variety of biologically active natural product analogues. With access to bulk quantities of the turimycin complex produced by *Streptomyces hygroscopicus* we have established a series of new analogues with promising biological activities.

Mutasynthesis represents another avenue to natural product derivatives that are not readily accessible through chemical synthesis. By

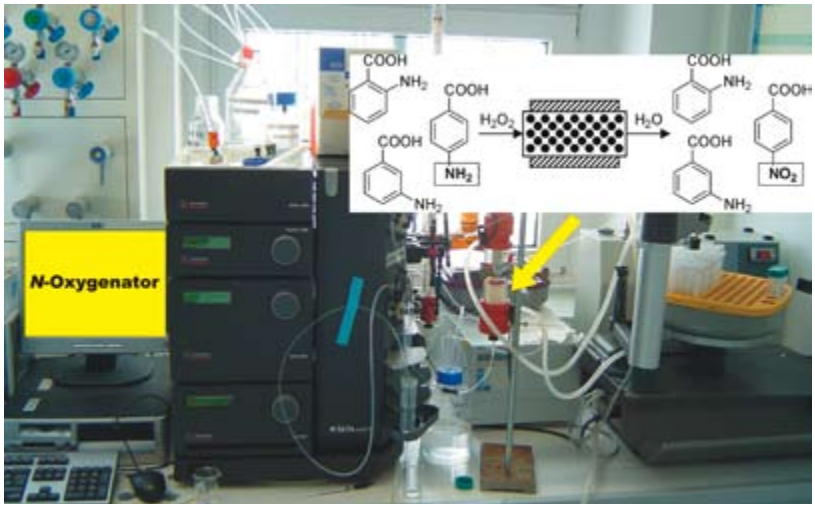



**Figure 4-1**
Continuous selective N-oxygenation by immobilized AurF.

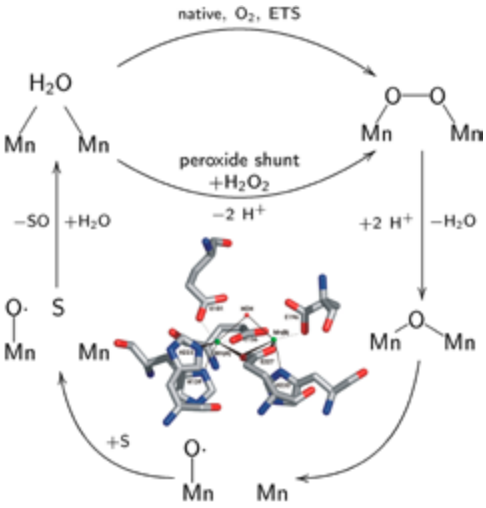



**Figure 4-2**
Binuclear manganese center of N-monooxygenase AurF and proposed catalytic cycle

means of this technique the natural biosynthetic pathway is suppressed by mutagenesis, which makes it possible to "force-feed" non-natural biosynthetic building blocks to the manipulated biosynthetic machinery. We have successfully explored the biosynthetic potential of bacterial polyketide synthases. For this purpose, suitable mutants were engineered. For complementation of the mutants, a number of non-natural building blocks were synthesized. (Details cannot be reported at this stage because of pending patents and publications.)

## 4 Biocatalysis

### Group Leader: Robert Winkler, Christian Hertweck

Biosynthetic pathways leading to complex secondary metabolites frequently involve enzymes with intriguing synthetic capabilities. A closer investigation of such enzymes may lead to novel biocatalysts that could complement the currently available synthetic repertoire.
We have studied a non-canonical oxygenase (AurF) that is capable to oxidize amino groups to nitro groups with high regio- and chemoselectivity. Preparative amounts of the enzyme were produced in *E. coli* and the catalytic cycle could be driven by $H_2O_2$. We have succeeded in the immobilization of the enzyme to selectively *N*-oxygenate the native substrate from a mixture of position isomers in a continuous flow, demonstrating the high potential for practical use (Fig. 4−1). The biochemical and structural characterization of the enzyme resulted in the discovery of a novel binuclear manganese cluster in the active site (collaboration Prof. Schulz, Freiburg). To explore the ligand sphere of the unique Mn centers we have performed nineteen structure-based rational site-directed mutagenesis experiments and tested the activity of the mutants. From these results we could clearly assign the essential residues in the complex for manganese coordination and

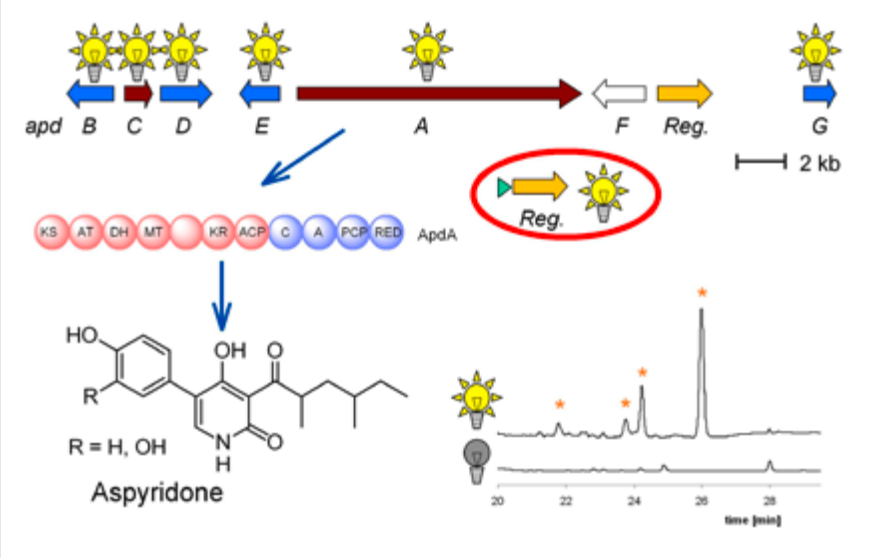



**Figure 5-1**
Controlled activation of the silent aspyridone biosynthesis gene cluster in *Aspergillus nidulans*.

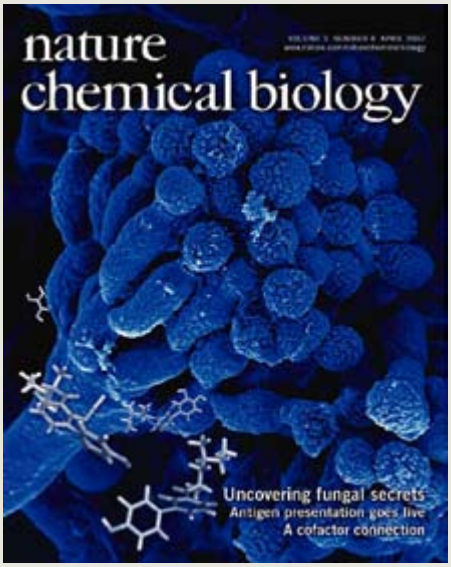



**Figure 5-2**
Nature Chemical Biology cover art

enzyme activity. In conjunction with the x-ray structure we could deduce the exact geometry of a novel dimanganese cluster that is capable of a regio- and chemoselective *N*-oxygenation. The involvement of Mn in catalysis and radical formation was monitored by ESR. On the basis of these results a mechanism according to cytochromes and binuclear Fe monooxygenases was proposed. This is the first report on the functional analysis of an Mn-dependent monooxygenase. The elucidation of this novel biocatalyst may guide the development of synthetic biomimetic oxygenation catalysts.

## 5 Genome Mining for Natural Product Discovery

Group Leaders: Kirstin Scherlach, Keishi Ishida, Christian Hertweck

Recent projects of whole genome sequencing of microorganisms revealed that the number of predicted biosynthesis genes is not reflected by the metabolic profile of these organisms observed under standard fermentation conditions. As these cryptic genes might code for the biosynthesis of important drug candidates or virulence factors new strategies are needed to make use of the biosynthetic potential of microorganisms.

Mining the genome of the model organism *Aspergillus nidulans* for cryptic biosynthesis genes we observed the presence of multiple anthranilic acid synthase gene copies. It is known that the gene products catalyze the transformation of chorismate to anthranilic acid, a key building block in the biosynthesis of tryptophane. However, the presence of multiple copies of putative AS genes prompted us to assume that some of their gene products might be involved in secondary metabolic pathways. Thus, the fungal metabolome was reinvestigated under various fermentation conditions, resulting in the discovery of novel

prenylated quinolin-2-one alkaloids, two of which bear unprecedented terpenoid side chains.

Furthermore, we discovered the presence of a putative PKS-NRPS hybrid gene cluster in the genome of *Aspergillus nidulans*. Investigation of the fungal metabolome implied that the gene locus is silent under laboratory culture conditions. In collaboration with the Department of Molecular and Applied Microbiology we established a strategy for the activation of the orphan PKS-NRPS gene cluster. Expression of a pathway specific regulatory gene under the control of an inducible promoter led to the induction of the fungal biosynthetic pathway (collaboration MAM). In consequence we were able to identify new secondary metabolites that exhibit cytostatic properties (aspyridone A and B). This is the first successful activation of a silent gene cluster in a eukaryote. The approach will be applicable to other natural product producers and allow the revelation of a hidden reservoir of bioactive metabolites.

Other examples of successful applications of genome mining involve the finding that the plant commensal *Pseudomonas fluorescens* Pf-5 is capable of producing antiproliferative and antifungal rhizoxin derivatives, the discovery of new aeruginosins from cyanobacteria (collaboration Prof. Dittmann, Berlin) and the artificial reconstruction of two cryptic angucycline antibiotic biosynthetic pathways (collaboration Dr. Metsä-Ketelä, Turku). We are currently exploring various microbial genomes for biosynthesis gene clusters for the identification of new, potentially bioactive compounds.

## 6 Microbial Interactions

Group Leaders: Laila P. Partida Martinez, Christian Hertweck

### Endofungal Bacteria

Stable symbiotic associations between endocellular bacteria and eukaryotes are well known in the animal and plant kingdoms and numerous groundbreaking studies have contributed to a deeper insight into the hallmarks and mechanisms of living together. Notably, only very little is known about symbiotic interactions between fungi and endobacteria, and only a few examples restricted to arbuscular mycorrhizal (AM) fungi have been discovered within the last decade. Only recently we found that such little explored symbioses also play a crucial role for the saprotrophic fungus *Rhizopus microsporus*, which is infamous for causing severe losses in rice nurseries. An antimitotic polyketide metabolite, rhizoxin, isolated from fungal cultures has been identified as the causative agent of the plant disease, known as rice seedling blight. We showed that this phytotoxin is not produced by the fungus, but by symbiotic bacteria of the genus *Burkholderia* that reside within the fungal cytosol. This case represents an unparalleled example for a symbiosis, in which a fungus harbors bacteria for the production of a virulence factor. Curing the fungus with an antibiotic, which resulted in a symbiont-free, rhizoxin-nega-

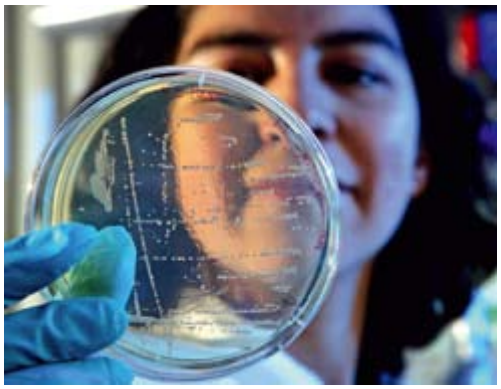


**Figure 6-1**
Laila P. Partida Martinez showing a plate with cultured symbionts

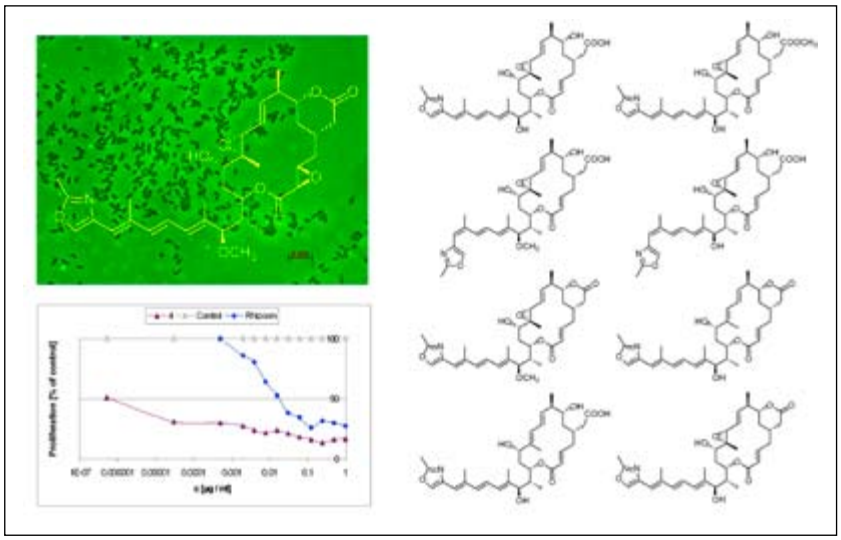


**Figure 6-2**
Endosymbionts in pure culture and selected structures of rhizoxin derivatives. Results from antiproliferative assay using rhizoxin (blue line) and derivative (purple line).

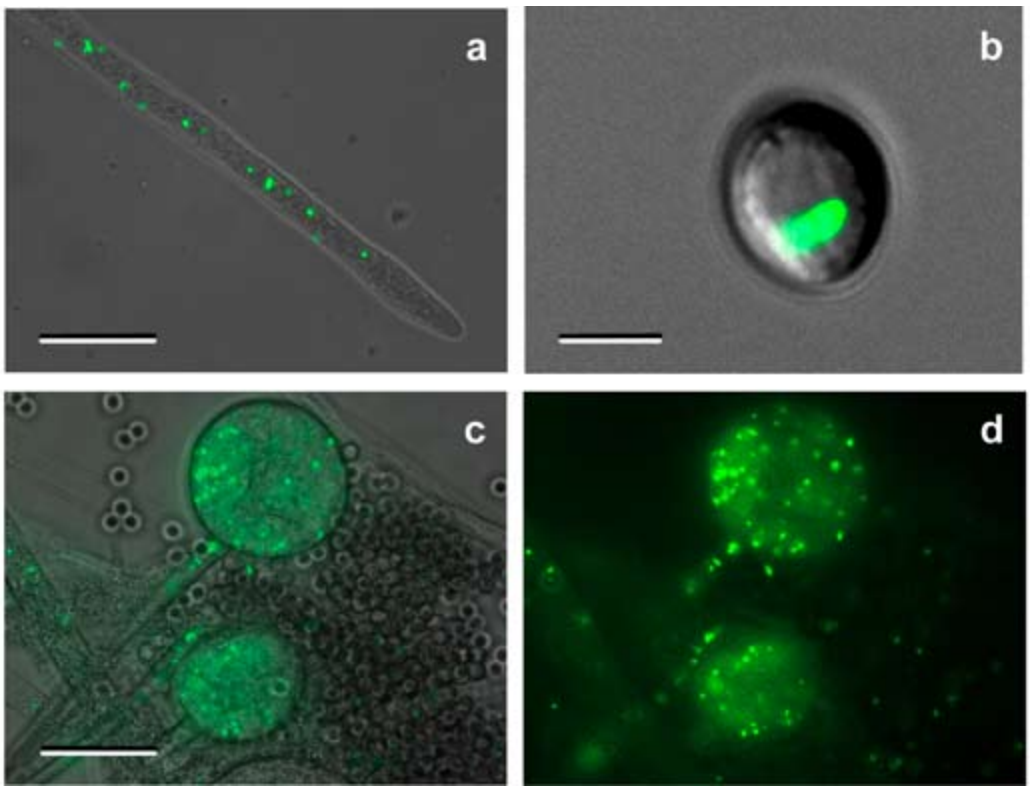



**Figure 6-3**
Confocal laser scanning micrographs of fungi after microinjection with GFP-labelled bacteria. Sporangiophores, sporangia and spores formed after reconstitution of the symbiosis.

tive phenotype, corroborated our findings. Furthermore, we succeeded in isolating the endosymbiont in pure culture and in proving its capability for rhizoxin production. Furthermore, he isolated symbiont produces a complex of over 20 rhizoxin analogues. To our surprise, some of the new metabolites are up to 10,000 times more active than rhizoxin (collab. IB, BT).

Cloning, sequencing and mutagenesis of the entire gene locus encoding rhizoxin biosynthesis within the symbiont genome revealed the molecular basis for toxin production. Finally, re-infection of the cured fungal strain with the isolated symbiont reestablished a rhizoxin-producing fungal-bacterial symbiosis.

More recently, we also found the second case where a mycotoxin is produced by endobacteria: the cyclopeptide rhizonin. Rhizonin has been termed the first reported mycotoxin from lower fungi and causes severe damages to the liver. As some *Rhizopus* species are used in the food industry, the identification of mycotoxins and their producers is highly important. Surprisingly, we could confirm that bacterial symbionts are the real producers of this peptide.

Screening of a collection of 22 *Rhizopus* strains revealed that only eight harbor bacterial endosymbionts. Each *Rhizopus* strain contains only one kind of bacteria and all of them form a new clade and belong to the Genus *Burkholderia*. Two of these endofungal bacterial strains underwent a full taxonomic description: *Burkholderia rhizoxinica sp. nov.* and *Burkholderia endofungorum sp. nov.* (collab. Dr. I. Groth).

The importance of this rare symbiotic bacterial-fungal interaction is highlighted by the unexpected observation that in the absence of bacterial endosymbionts the fungal host is not capable of vegetative reproduction. Formation of sporangia and spores is only restored upon

re-introduction of endobacteria. The fungus has become totally dependent on endofungal bacteria, which in return provide a highly potent toxin for defending the habitat and accessing nutrients from decaying plants.

Genomic data have been generated for both *Burkholderia rhizoxinica* and *Burkholderia endofungorum* and will support our understanding of the complex biochemical processes taking place in these symbiotic bacteria.

### Saprotrophic Tree Fungi and Associated Microorganisms

In the past years we have identified a number of anti-inflammatory compounds from tree fungi. By bioassay-guided isolation, phenylpropanoid-derived polyketides, including an unusual 5-methyl-3(*2H*)-furanone derivative (inotilone) with potent cyclooxygenase (COX) and xanthone oxidase (XO) inhibitory activities were obtained from the fruiting body of the mushroom *Inonotus sp.* Two novel fungal phenylpropanoid-derived polyketides, squarrosidine and pinillidine, with potent XO inhibitory activities were isolated from the fruiting bodies of the mushroom *Pholiota squarrosa* and from the mycelium of *Phellinus pini*. Structural analyses revealed that both metabolites from the tree fungi represent unprecedented 3,3'-fused bis-styrylpyrones. We were also intrigued by the metabolic capabilities of the wood-rotting basidiomycete *Daedalea quercina* (commonly known as Oak Mazegill). This wood decay fungus, which generally grows on living oaks, appeared to be well equipped to neutralize first line defense chemicals of the plant. We have identified quercinol, a new member of the chromene family of natural products, as the active principle. In various assays using enzymes that are commonly involved in inflammation processes and oxidative burst, quercinol showed inhibitory activities at micromolar concentrations. It is tempting to speculate about the role of quercinol in its natural context. Obviously the fungus, which infects pruning wounds of oak trees, can cope with the hypersensitive response. Considering the high potency against ROS quercinol might serve the fungus to dampen the plant defense mechanism.

In the course of these studies we were surprised to find an *Epicoccum* species growing within the fruit body of the saprotrophic tree fungus *Pholiota squarrosa*. We have succeeded in cultivating the fungus and characterized three new polyketide-amino acid hybrid metabolites, the potent antibacterial tetramic acid epicoccarines A and B, the pyridone compound epipyridone, and three new members of the tetramic acid family of natural products.

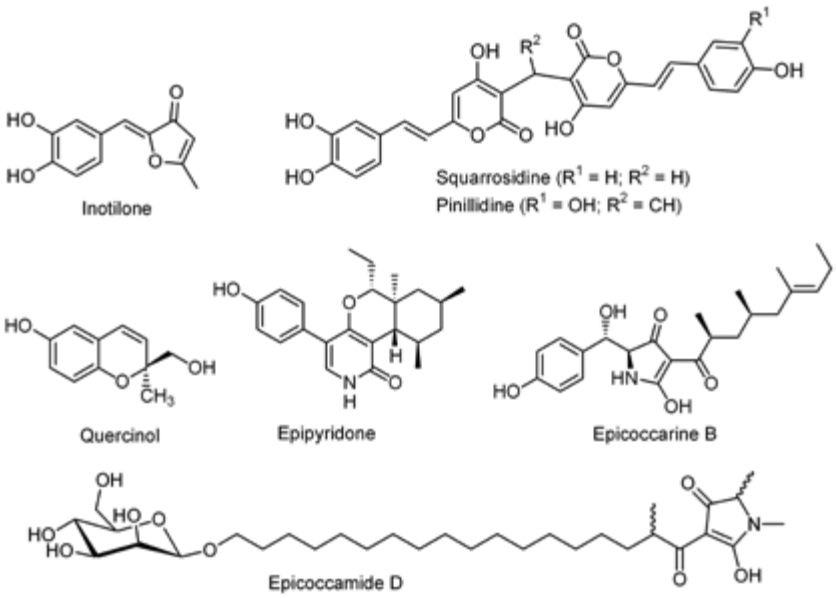



**Figure 6-4**
Selected structures of novel compounds isolated from tree fungi and associated *Epicoccum* sp.

## 7 Mass Spectrometry Based Protein and Peptide Analysis

Group Leader: Robert Winkler

The department of Biomolecular Chemistry operates the institute's peptide and protein analysis platform. The department is equipped with a MALDI-TOF/TOF, an ESI-QTOF and a nanoLC-ESi-ion trap with ETD option (Fig. 7-1). Up to 200 proteomics samples can be measured per day on the MALDI-TOF/TOF. The analysis of the data is performed on in-house servers, which also allows the usage of unpublished genomic databases. We have established a set-up that allows the measurement of proteomic samples with extremely low peptide concentrations or peptide mixtures by nanoLC-MS. This technique has been applied for the identification of proteins originating from sepsis patients. In addition, we have studied protein and peptide

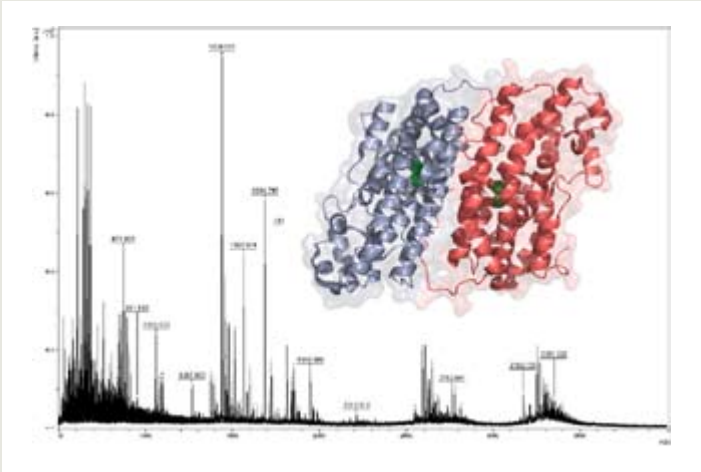


**Figure 7-1**
Mass spectrometers used for protein and peptide analysis: ESI-QTOF, nanoLC-MS and MALDI-TOF/TOF

**Figure 7-2**
Mass spectrum of a tryptic protein digest to search for covalently bound cofactors (protein: AurF)

modifications, such as protease degradation of peptide antibiotics, phosphorylation/dephosphorylation, cofactors of proteins (Fig. 7-2), as well as artificial modification of proteins, like biotinylation of antibodies. For quick identification of microorganisms we now also use a modern MALDI-TOF based approach. The protein mass spectra are typical for each organism, because of its ribosomal and housekeeping proteins. Therefore it is possible to train a database and to use it later for rapid detection. We have employed this technology e.g. for at-line fermentation control when culturing slowly growing microorganisms that easily get overgrown by other microorganisms. The method also helped revealing phylogenetic relationships between microorganisms and to easily remove redundancies from strain collections.

## 8 Natural Products Screening (and Compound Collection)

Group Leader: Isabel Sattler

### Microbial Natural Products - Isolation, Structure Elucidation and Supply for Application in Drug Discovery and Biotechnology

In 2006, the research group “Natural Products Discovery” was integrated into the department “Biomolecular Chemistry”. The research group focuses on the exploitation of structural diversity from a broad spectrum of natural sources for providing new compounds for understanding biosynthetic capacities of the producing organisms and for biological characterization, e.g. in drug discovery (Fig. 8-1). We are relying on a number of different resources for bioprospecting. In the last two years, our major natural products screening programs were run on:

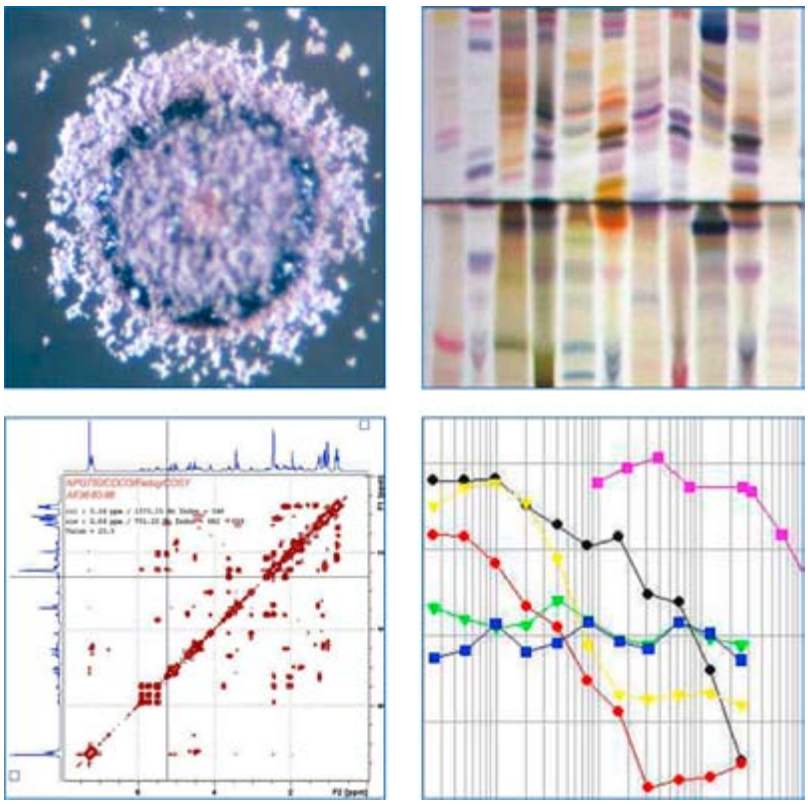


**Figure 8-1**
Chemical screening for novel bioactive natural products

(i) Extremophilic and rare actinomycetes, as well as filamentous fungi, from unusual habitats from the southwest of China (provided by Key Laboratory for Microbial Resources, Yunnan University, Kunming, P.R. China),

(ii) Fungi from cold marine habitats (provided by Alfred-Wegener-Institut für Polar- und Meeresforschung, Bremerhaven, Germany)

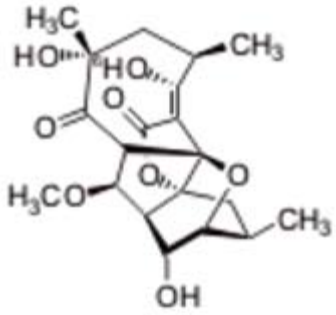
(iii) Endophytes from mangrove plants from southeast China (plant material provided by National Research Laboratories of Natural and Biomimetic Drugs, Peking University Beijing , P.R. China)

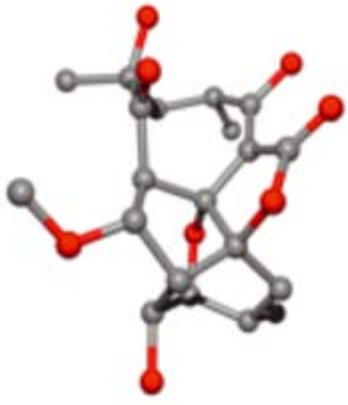
(iv) Rare actinomyces from heavy-metal contaminated habitats, e.g. from the Feengrotten, a former alum slate mine near Saalfeld (Thuringia) and former uranium mining area around Ronneburg, Thuringia (together with Institute of Microbiology, Friedrich Schiller University of Jena)

In the search for new natural products, we efficiently intertwine biologically driven approaches, e.g. antimicrobial and cytotoxicity screening, with the physico-chemical analysis of the complex compound mixtures extracted from the producing organisms. Combined with the particular scientific objectives of our projects, we continuously thrive to optimize our screening procedures in order to improve methods for the effective exploitation of natural products. A particular focus on advancing our screening routines is set on improving dereplication by HPLC/$MS^n$-analysis, including a database with HPLC/UV/MS data of over four thousand natural products, and HPLC/MS-driven separation on the analytical and preparative scale. For biological testing we rely on our colleagues of the IPL team and external collaboration partners. Our major goal to access meaningful, and best, structurally new, compounds in the subsequent chemical projects including chromatographic isolation and structure elucidation.

**Figure 8-2**
Chemical structures of shearinines D-K **(1-8)**, shearinine A **(9)**, paspalitrem A **(10)**, and paspaline **(11)**. Shearinines D-K **(1-8)** are new natural products produced by *Penicillium sp.* (HKI0459), an endophytic fungus of the mangrove tree *Aegiceras corniculatum (Aegiceratataceae).*


**Figure 8-3**
Structural diagram and x-ray structure of the polycyclic *Streptomyces* metabolite abyssomicin E.



In our screening program on endophytic microorganisms form mangrove trees, *Penicillium sp.* strain HKI0459, isolated from the stems of *Aegiceras corniculatum* (*Aegiceratataceae*), was investigated. Pilot scale cultivation (200 L, by HKI Bio Pilot Plant) of this fungus resulted in the isolation and characterization of eight new indole triterpenes named shearinines D-K (**1-8**), along with shearinine A (**9**), paspalitrem A (**10**), and paspaline (**11**) (Fig. 8-2). The stereostructures of the new compounds were determined by extensive spectroscopic data analyses and quantum chemical circular dichroism calculations.

The indole alkaloids of the janthitrem class and related compounds are a small group of about 50 fungal metabolites with a unique polycyclic skeleton. The newly found shearinines D (**1**) to K (**8**) carry some structural features that were not known within this class of compounds before. Based on increasing structural complexity, the compounds identified in this study were fitted into a hypothetical biosynthetic grid that provided new insights into the biosynthesis of the shearinine/paspalitrem-type janthitrem indole alkaloids such as shearinine A (**9**) possibly serving as a missing link that was before only hypothetically proposed. In biological activity profiling, shearinines D (**1**), E (**2**), and (with reduced potency) G (**4**) exhibited *in vitro* blocking activity on large-conductance calcium-activated potassium channels in the nanomolar range. The BKCa channel is implicated in various diseases, including epilepsy, hypertension or erectile dysfunction. Our data show that the substituents of C-22, similar to those of C-13,23 are important determinants for BKCa inhibition by janthitrems. As proposed for other tremorgenic mycotoxins, BKCa blocking shearinines like 1 and 2 may cross the blood-brain barrier and, thus, could be of therapeutical value for treatment of special forms of epilepsia.isa4 Besides, they may be helpful tools for specifically modifying BKCa calcium- and voltage-dependent gating processes, which are still not fully understood.

Abyssomicins are highly functionalized polycyclic metabolites with a unique C19 skeleton. We have isolated abyssomicin E from *Streptomyces* sp. (HKI0381) and fully elucidated its structure on the basis of NMR and MS data. Moreover, for the first time the absolute stereochemistry was directly established through the application of anomalous dispersion with copper radiation in the single-crystal X-ray diffraction study (Fig. 8-3).

### Natural Products Pool

The compound collection "Natural Products Pool" that comprises about 9,000 samples from more than 90 partners worldwide and is maintained by the project group Natural Products Screening. After 10 years of mainly industrial based drug discovery, compound libraries of various sizes are currently solely supplied to academic research groups. With the aim of drug discovery and chemical genomics three research groups entered the consortium in 2006/2007 for testing in a wide variety of applications, e.g. novel targets in the search of antimalarials (Professor Becker, Justus-Liebig-Universität Giessen). Thus, several hundred compounds were tested on chloroquine resistant malarial parasites *in vitro* as well as against some of the currently most attractive antimalarial drug targets including glutathione reductase, thioredoxin reductase, glucose-6-phosphate dehydrogenase and glutathions S-transferase. *In vivo* analyses in the mouse model and studies to optimize the respective lead compounds are under way. In a screening program using novel RNA-aptamer competing screening technology, the triterpene quercinic acid was identified as a competitive binding agent in the nanomolar-range to a Trypanosome surface-specific aptamer. Thus, the Natural Products Pool might enable the development of novel therapeutics for diagnostics and therapy of sleeping sickness.

The HKI Natural Products Pool enabled the identification a series of fatty acylated benzamido inhibitors of the cis/trans isomerase activity of DnaK. The hsp70 chaperone DnaK from *E. coli* might assist protein folding by catalyzing the cis/trans isomerization of secondary amide peptide bonds in unfolded or partially folded proteins. The compounds were shown to compete with fluorophore-labeled $\sigma$32-derived peptide for the peptide-binding site of DnaK and to increase the fraction of aggregated proteins in heat-shocked bacteria. Thus, it could be shown that the isomerase activity of DnaK is a major survival factor in the heat shock response of bacteria and that small molecule inhibitors can lead to functional inactivation of DnaK and thus will display antibacterial activity.

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Bundesministerium für Bildung und Forschung
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Teilprojekt: Neue antibakterielle und antitumorale Polyketide durch Biokombinatorik
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Teilprojekt: Neue biologisch aktive Substanzen aus chinesischen Heilpflanzen und Schwämmen und ihren assoziierten Mikroorganismen
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Teilprojekt: Die nicht-colinearen Aureothin- und Neoaureothin-Biosynthesewege als Modellsystem für die Evolution von Polyketidsynthasen
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European Union
5. Forschungsrahmenprogramm der EU,
Schwerpunktthema: Quality of Life and Management of Living Resources
Projekt: Eukaryotic polyketides in surrogate hosts
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Focusing on Hormone-Independent Breast Cancer
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(HKI authors in bold)


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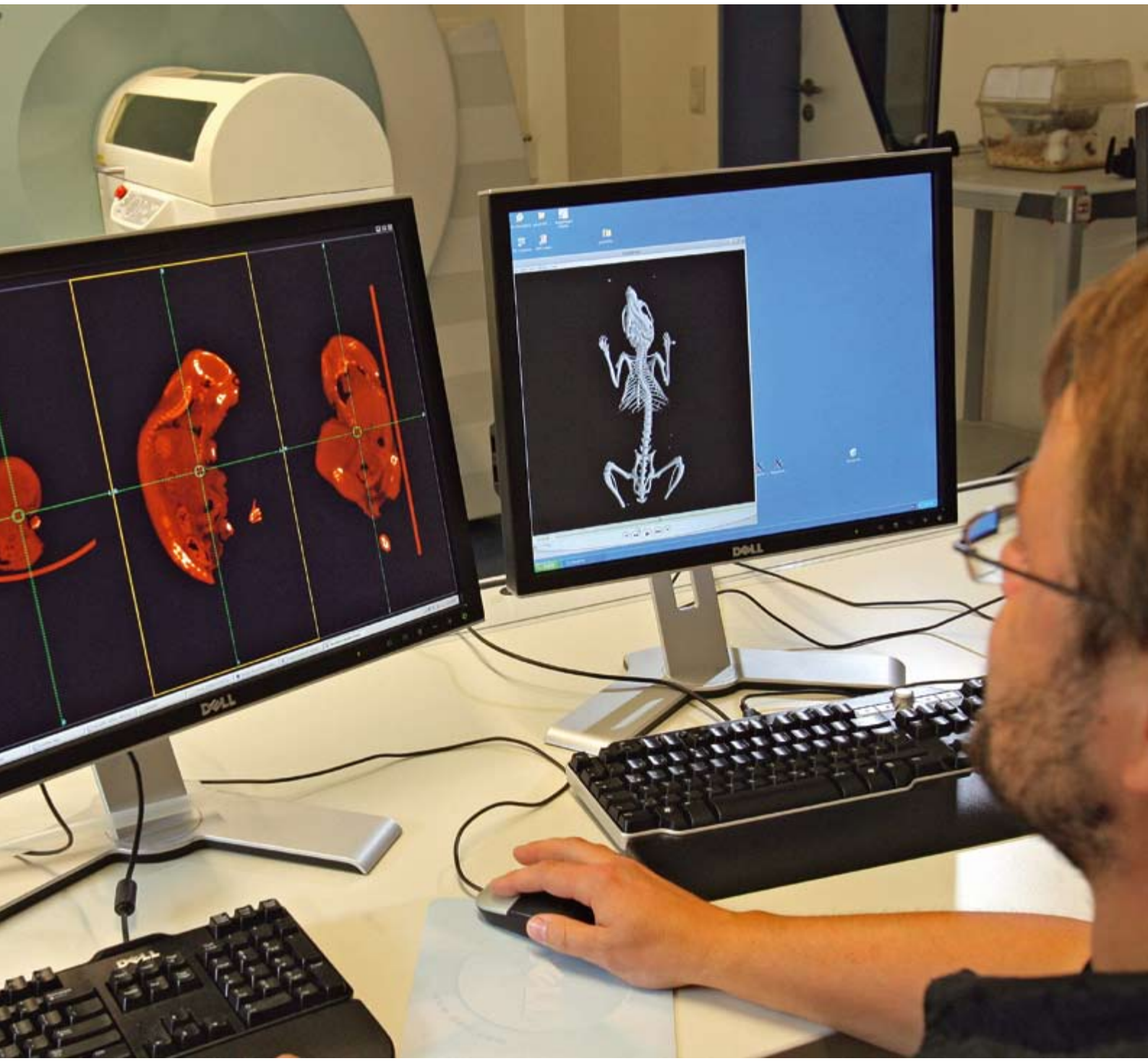



# Department of Cell and Molecular Biology

# Department of Cell and Molecular Biology

Research in the Department of Cell and Molecular Biology is devoted to the study of stress-related host-response reactions like apoptosis, which arise naturally by interactions between man and pathogenic microorganisms or by physical means. To that end we have set out to adopt and develop highly advanced micro- and nanosytems, which allow the simultaneous handling of thousands of samples within sets of different biomolecules under nearly identical experimental conditions. At present we are focussing on parallel Rapid PCR, chip/array technologies and biopolymer-interaction technologies.

Those micro- and nanosystems are also ideally suited for application in other departments of our institute, as well as within the entire "Beutenberg Campus" in Jena, where research interests in such different fields as physics, chemistry and biology meet. Combined with the automation of techniques, these systems will also provide effective tools for the rapid realization of products and instruments – an important aspect in the context of the "BioRegio" Jena network.

To have optimum access to most advanced scientific know-how and technical equipment we cooperate with several institutions and industries locally and internationally. Experience and knowledge gained from our projects allow us to teach and confront students at university theoretically and practically with

## INTRODUCTION | EINLEITUNG

**Head:**
**Prof. Dr. Hans Peter Saluz**

Die Abteilung Zell- und Molekularbiologie bearbeitet schwerpunktmäßig stressbedingte Wirtszellreaktionen wie Apoptose, die natürlicherweise als Folge von Interaktionen zwischen Wirtszelle und Pathogen oder physikalischer Einwirkungen auftreten. Um die verschiedenen, wirts-spezifischen Antworten wirkungsvoll erfassen zu können, sind wir dabei, hochmoderne Mikro- und Nano Systeme anzupassen oder neu zu entwickeln. Mit ihnen können jeweils mehrere tausend Proben aus einer Reihe unterschiedlicher Biomoleküle gleichzeitig und unter nahezu identischen Versuchsbedingungen untersucht werden. Zur Zeit beschäftigen wir uns mit Paralleler Rapid PCR, Chip/Array-Technologien und multiplen Hybridsystemen.

Mikro- und Nanosysteme eignen sich auch zur Anwendung in anderen Abteilungen unseres Institutes, sowie auf dem gesamten Beutenberg Campus in Jena, wo die Forschungsinteressen aus so verschiedenen Gebieten wie Physik, Chemie und Biologie in einzigartiger Weise aufeinander treffen. Außerdem liefern diese Systeme, kombiniert mit der Möglichkeit zur Automatisierung, eine Voraussetzung für die effiziente Realisierung von Produkten und Instrumenten, für die „BioRegio" Jena ein nicht unwesentlicher Aspekt.

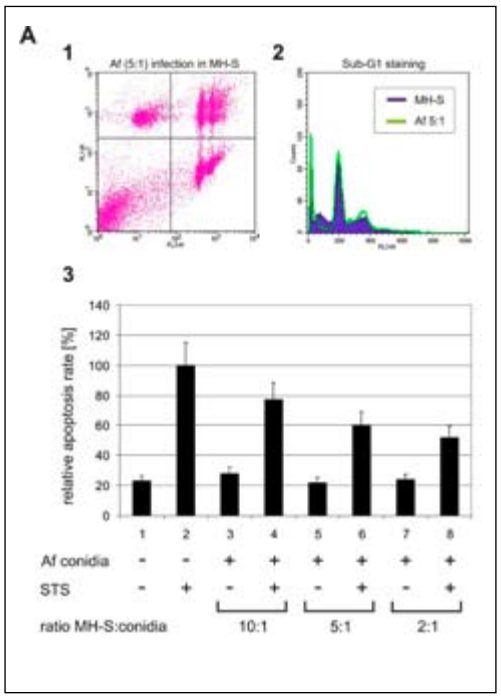
Um einen optimalen Zugriff auf neueste wissenschaftliche Erkenntnisse und technische Mittel zu haben, kooperieren wir mit mehreren lokalen und internationalen Instituten und Unternehmen. Die Erfahrungen und Kenntnisse, die wir aus unseren Arbeiten gewinnen, erlauben es uns, Studenten theoretisch und praktisch mit modernsten Aspekten der Grundlagenforschung und angewandten

modern aspects of basic and applied research. During the last two years several diploma and doctoral students have graduated successfully at our department. Within our technological framework we have had some real success on two patent families (totally approx. 20 patents/applications) related to rapid heat block thermocycling of small samples. The issuance of these internationally coveted patents reinforces the HKI patent portfolio in the area of advanced technologies.

The ability to rapidly amplify nucleic acids is particularly important for pathogen detection in clinical diagnostic applications (especially in near-patient testing), as well as in life science research and industrial applications. Two commercial devices, i.e. the SpeedCyler and corresponding consumables, are produced and sold worldwide by Analytik Jena.

Wissenschaft zu konfrontieren. So hatten wir in den letzten zwei Jahren wiederum mehrere Diplomanden und Doktoranden in unserer Abteilung, am FLI und am Max Planck Institut in Jena, die ihre Studien mit Erfolg abschliessen konnten.

Was unsere technischen Projekte anbetrifft, konnten wir guten Erfolg in Hinblick auf Rapid PCR verzeichnen, da unsere international begehrten Erfindungen allgemein anerkannt wurden und die Patentfamilien (z. Z. ca. 20 Patente/Anmeldungen) das HKI-Portfolio zum Thema „Hochtechnologien“ verstärken. Die Möglichkeit, Nukleinsäuren sehr schnell zu amplifizieren ist wichtig für Pathogenidentifikationen in der medizinischen Diagnostik, spielt aber auch in der biologischen Forschung und Industrie eine zentrale Rolle. Zwei verschiedene Geräte der sogenannten SpeedCycler Familie mit dazugehörenden Verbrauchsmaterialien werden zur Zeit von Analytik Jena auf Grund unserer Patente hergestellt und weltweit vertrieben.



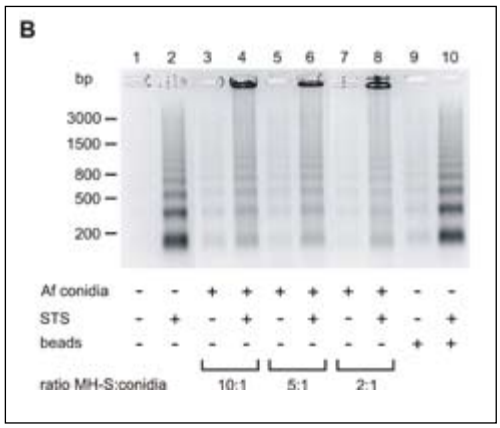




**Figure 1**
**Apoptosis of MH-S alveolar macrophages exposed to Af conidia.**
**A)** Flow cytometry analysis of MH-S cells exposed to Af conidia and treated with STS. Propidium Iodide (PI) fluorescence (FL-3) is presented on the horizontal axis and FITC fluorescence (FL-1) on the vertical axis. (1) The lower right quadrant of the cytogram represents the MH-S cells ($PI^+/FITC^-$) while FITC-labeled Af conidia were recorded in the upper left quadrant ($FITC^+/PI^-$) and the upper right quadrant represents MH-S cells with phagocytosed Af conidia ($PI^+/FITC^+$). In each case, the fluorescence of 10000 cells was assessed. (2) Merged histograms of MH-S cells and MH-S cells infected with Af conidia (Ratio MH-S:conidia = 5:1) and treated with 1.5 µM STS for 4 h. (3) Inhibition of apoptosis depends on the amount of conidia. The apoptosis rate was calculated with data from three experiments. +, presence; -, absence of STS or conidia.

**B)** Fragmentation patterns of DNA from MH-S cells exposed to Af conidia and treated with 1.5 µM STS for 4 h. DNA fragmentation was down regulated in cells treated with different concentrations of Af conidia and STS in a dose-dependent manner. As a positive control, MH-S cells were exposed to 1 x $10^6$ Align Flow PLUS cytometry beads (Molecular Probes, Eugene, Oregon, USA). In repeated experiments, similar results were obtained. +, presence; -, absence.

## 1 Cellular Stress and Apoptosis

Group Leader: Hans Peter Saluz

### Apoptosis Inhibition of Alveolar Macrophages upon Interaction with Conidia of *Aspergillus fumigatus*

Infections with mould pathogens have emerged as an increasing risk faced by patients under sustained immunosuppression. Species of the *Aspergillus* family account for most of these infections and in particular *Aspergillus fumigatus* (Af) can be regarded as the most important airborne-pathogenic fungus (Latgé, 1999). The improvement in transplant medicine and the therapy of hematological malignancies is often complicated by the threat of invasive aspergillosis. Specific diagnostics are still limited, as are the possibilities of therapeutic intervention. Hence, invasive aspergillosis is still associated with a high mortality rate that ranges from 30 % to 90 % (Baddley et al., 2001; Denning, 1998). Recognition of invading microorganisms by the innate immune system is a first and essential step in their successful elimination (e.g. Schaffner et al., 1982; Behnsen et al., 2007). Alveolar macrophages are the major resident cells of the lung alveoli; they, along with neutrophils (which are actively recruited during inflammation), are the major cells in the phagocytosis of Af (reviewed in Romani, 2004; Brakhage, 2005). There is little data on the processing of conidia by the host and the effect of conidia on apoptosis yet. Many extra- and intracellular pathogens evolved different mechanisms to escape host cell suicide by directly interfering with apoptotic pathways (reviewed in Luder et al., 2001; Muller and Rudel, 2001). Therefore, in this study we have investigated interactions between *A. fumigatus* conidia and alveolar macrophages, one of the major defence immune effector cells against this pathogen. Within the framework of this project we could demonstrate that *A. fumigatus* inhibits host cell apoptosis of alveolar

macrophages (Fig. 1) This unexpected result was due to inhibition of caspase 3 by a yet unknown mechanism (Volling et al., 2007).

## 2 The Preferentially Expressed Antigen of Melanoma (*PRAME*): Regulation and Role in Apoptosis

Group Leader: Hans Peter Saluz

### Apoptosis

Within the framework of this project we investigate regulation and function of the preferentially expressed antigen of melanoma (*PRAME*) which encodes a protein recognised by autologous cytolytic T lymphocytes. The mRNA level of *PRAME* is used as a tumour marker due to its overexpression in various malignancies. On the other hand, overexpression of genes encoding antiapoptotic proteins leads to the survival of leukaemic cells via exclusion of apoptosis. Therefore, we investigate the relationship between *PRAME* overexpression and the expression of apoptosis-related genes in childhood *de novo* AML patient samples and, furthermore, whether this is a general or an AML-subtype specific event.

The preferentially expressed antigen of melanoma (*PRAME*) is one of the most important Cancer/testis-associated genes (CTAs). CTAs are a subgroup of tumor antigens which are predominantly expressed in testis and a variety of cancers. Therefore, they are clinically used as tumor markers and targets for immunotherapy of human malignancies. Although only some of them are functionally characterized most seem to play a role in cell cycle regulation or transcriptional control.
*PRAME* was identified as a gene encoding an HLA-A24–restricted antigenic peptide which is recognized on a human melanoma cell line by a specific autologous CTL clone. In contrast to healthy people, where *PRAME* expression is only detectable in testis and to a much lower degree in endometrium and ovary, it is expressed at a high level in large fractions of malignant deteriorations like melanomas, non-small-cell lung carcinomas, head and neck squamous carcinomas, sarcomas, renal carcinomas and others. In contrast to other tumor-associated antigens, it is also expressed in leukemia. It was found among others in 42 % of 98 AML-patients (Matsuhita, 2001), 62 % of 31 childhood AML patients, 42 % of 21 childhood ALL patients (Steinbach, 2003) and 26 % of 58 patients with CLD (Proto-Siqueira, 2003). Therefore, *PRAME* is a well suited tumor marker for many tumor types and the probably most valuable CTA in leukemia. Furthermore, due to its significant reduction in patients upon chemotherapy, it was also shown to function as a useful parameter for monitoring minimal residual disease (MRD) (Matsuhita, 2001).

We performed detailed microarray experiments involving approximately 300 genes relevant for cell cycle control and apoptosis. All together 27 clinical samples of *de novo* childhood AML, including subtypes (M1/2, M4 and M5) were investigated. The study revealed 18 (6 %) up- and 13 (4 %) down regulated genes correlating with *PRAME* over expression. A set of differentially expressed genes was reconfirmed by RT-PCR.

In parallel to the above studies we have investigated regulation of the *PRAME* gene itself in cancer cell lines, blood from healthy people and AML tumour samples (Schenk et al., 2007). *In vivo* genomic sequencing experiments (Saluz and Jost, 1989) revealed epigenetic regulation through specific changes in DNA cytosine methylation. The data could be confirmed by methylating corresponding DNA *in vitro* followed by transfection experiments. Vast majority of single CpG dinucleotides is methylated in eukaryotic genomes whereas CpG islands tend to be undermethylated. Methylation pattern can vary in both temporal and spatial manner. Promoters, which are embedded in CpG islands can be differentially regulated by this mechanisms since certain transcription factors selectively bind to methylated or unmethylated CpG dinucleotides within their consensus sequences.

Furthermore, we treated HeLa and Caski cells with the histone deacetylase inhibitors valproic acid (VPA) and trichostatin A (TSA). *PRAME* expression could be increased by TSA

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to a similar extend as obtained by 5-aza-dC. This result provides further evidence of the epigenetic regulation of the *PRAME* promoter since transcription factors and methyl-CpG-binding proteins interact with various classes of histone deacetylases (HDACs) in complexes, which can repress transcription and possibly induce methylation.

## 3 Apoptosis-related Events in Human Cells Treated with Fungal Anti-cancer Compounds

Group Leader: Hans Peter Saluz

### Cellular Stress and Apoptosis

The major aim of this study concerns differential gene expression and functional protein analysis of specific pathways like apoptosis in human cells upon treatment with well characterized bioactive anti-cancer compounds. The compounds have been isolated from Asian medical fungi such as Poria cocos and subsequently used for the treatment of suitable human cells. The potential action of the bioactive compounds on the regulation of human gene expression is analysed by array/chip-technologies which are well established at the department. Our special interest concerns inflammatory and/or apoptotic pathways studied by topic-related subarrays using our human cDNA libraries. Initially 75.000 human cDNA-clones have been used to select approximately 10.000 characterized stress inducible genes. In addition, it is planned to investigate the function of proteins (Westerns, MALDI-TOF, etc) encoded by the most relevant differentially expressed genes involved in the action of the bioactive compounds used.

Many of the available anti-cancer agents are derived from natural products, e.g. paclitaxel (Taxol), and camptothecin (Hycamtin) and many others. One had become aware that these natural products were being used extensively in Far East for the development of pharmaceutical-grade medicines to treat many different diseases, including cancer. The substantial range of medicinal fungi from which different bioactive compounds can be derived suggested that they could be used as a source of novel anti-cancer agents. Numerous bioactive fungal polymers are described that they somehow innate cell-mediated immune responses, and exhibit antitumour activities in animals and humans (Pelley et al., 2000). Stimulation of the host immune defence systems by bioactive polymers from medicinal fungi has significant effects on the maturation, differentiation and proliferation of many kinds of immune cells in the host (Fisher et al., 2002; Silva et al., 2002). Whilst the molecular mechanism of antitumour actions is still not understood, stimulation and modulation of host immune responses by such polymers appears central (Borchers et al., 1999). Therefore, we investigate differential gene expression and functional protein analysis of specific pathways in human cells upon treatment with fungal bioactive anti-cancer compounds. The compounds have been isolated from Asian medical fungi such as Poria cocos and subsequently used for the treatment of human cell lines derived from human cervix carcinoma (Hela), human hepatocellular carcinoma (Hep-G2), squamous carcinoma (CLS-354) and human breast carcinoma (MDA-MB 436) (strains are signed as E1, H1, H3, N1, and P1). Initially, treated cell lines were subjected to classic tests in order to gain more information on apoptosis, cell toxicity and antiproliferation. In all cell lines tested no cytotoxic effects could be observed. Upon treatment with ethanolic and aqueous extracts, a concentration-dependent inhibition of cell proliferation was obtained and cells developed many of the hallmark features of apoptosis. At high concentration, some of cell lines expressed antiproliferative effects. Chemical analysis revealed polysaccharides of different chemical composition, most of which belonged to the group of β-glucans, these have β-(1→3) linkages in the main chain of the glucan and additional β-(1→6) branch points that are needed for antitumor actions. Such polysaccharides are described to prevent oncogenesis and to show antitumor activity against various allogeneic and syngeneic tumors. At present we are fabricating special DNA microarrays to investigate above observations on a molecular level. For this we focus on differential expression of genes involved in inflammatory and/or apoptotic mechanisms. The proteins which are

encoded by the most relevant differentially expressed genes will be subjected to functional studies.

## 4 Human DNA Topoisomerase IIβ Binding Protein 1 (TopBP1) and Cell Division Cycle Protein 45 (Cdc45) at the Initiation Point of Replication

Group Leader: Frank Hänel

### Replicative Stress

TopBP1 was initially identified as a DNA topoisomerase IIβ-interacting protein. Human TopBP1 possesses eight BRCA1 carboxyl-terminal (BRCT) domains, a motif which was first described at the C terminus of the breast cancer susceptibility gene product, BRCA1, and which is conserved in many proteins related to the cell cycle checkpoint and DNA damage response. TopBP1 shares sequence homology with *Saccharomyces cerevisiae* Dbp11, *Schizosaccharomyces pombe* Rad4/Cut5, *Drosophila melanogaster* Mutagen-sensitive-101 (Mus101), and *Xenopus laevis Xmus* 101/Cut5. Like their yeast counterparts, human TopBP1 is required for cell survival, DNA replication, resistance to DNA damage and checkpoint control. The literature on TopBP1 also suggests a function as a transcriptional regulator.

While it is clear that human TopBP1 participates in the DNA damage checkpoint control, in human cells, however, the role of TopBP1 in DNA replication or S phase progression is poorly understood. The human transcript encoding TopBP1 and TopBP1 protein levels both increase during S phase, suggesting involvement in replication. Neutralizing TopBP1 with a polyclonal antiserum raised against the sixth BRCT-repeat inhibited replicative DNA synthesis in HeLa cell nuclei *in vitro*. Replication was inhibited more effectively by the recombinant protein fragment itself. This may indicate that the sixth BRCT domain is critical for replication activity, possibly interacting with a crucial replication factor. Moreover, Jeon and co-workers very recently showed, that human TopBP1 is required for G1 to S progression in a normal cell cycle. TopBP1 deficiency inhibited cells from entering S phase by up-regulating p21 and p27, resulting in down-regulation of cyclin E/CDK2 (Jeon et al., 2007).

Yeast Rad4/Cut5$^{TopBP1}$ and Dbp11$^{TopBP1}$ are essential for the initiation of DNA replication. Our detailed knowledge of replication initiation has been established from work realized mainly in the budding yeast and *Xenopus* systems. During G1 phase, origin recognition complex (ORC), Cdt1, Cdc6 and the minichromosome maintenance proteins 2-7 ($MCM_{2-7}$) proteins are sequentially assembled on the origin to form a pre-replicative complex (pre-RC). Then the pre-RC is converted to an initiation complex (IC) at the onset of S phase by S-phase cyclin dependent kinases (S-CDKs) and the Cdc7-Dbf4 kinase (DDK). This leads to the binding of Cdc45 and to the unwinding of the DNA at the replication origin. Cdc45 is an essential replication factor that has been reported to load the initiating DNA polymerase α-primase complex, and to act as a processivity clamp for the putative DNA helicase MCM 2-7. Furthermore, human Cdc45 may play an important role in elongation of DNA replication by bridging the processive DNA polymerases δ and ε with the replicative helicase in the elongation machinery. In *Xenopus*, Xmus101/Cut5 protein is required for the recruitment of Cdc45 to origins of DNA replication (Machida et al., 2005).

To improve our understanding of the physiological functions of human TopBP1 we investigated the interplay between human TopBP1 and Cdc45 proteins in synchronized HeLa-S3 cells. Using glutathione S-transferase (GST) pull-down and co-immunoprecipitation techniques we showed a direct interaction between TopBP1 and Cdc45 *in vitro* and *in vivo*. The use of deletion mutants in GST pull-down assays identified the first and second as well as the sixth BRCT domains of TopBP1 to be responsible for the functional interaction with Cdc45. Moreover, the interaction between Cdc45 and the first and second BRCT domains of TopBP1 inhibited their transcriptional activation both in yeast and mammalian one-hybrid systems. Both proteins interacted exclusively at the G1/S boundary of cell cycle, only weak interaction could be found at the G2/M boundary. The overexpression of the sixth BRCT domain led to diminished loading of Cdc45 onto chromatin. These data suggest that human TopBP1


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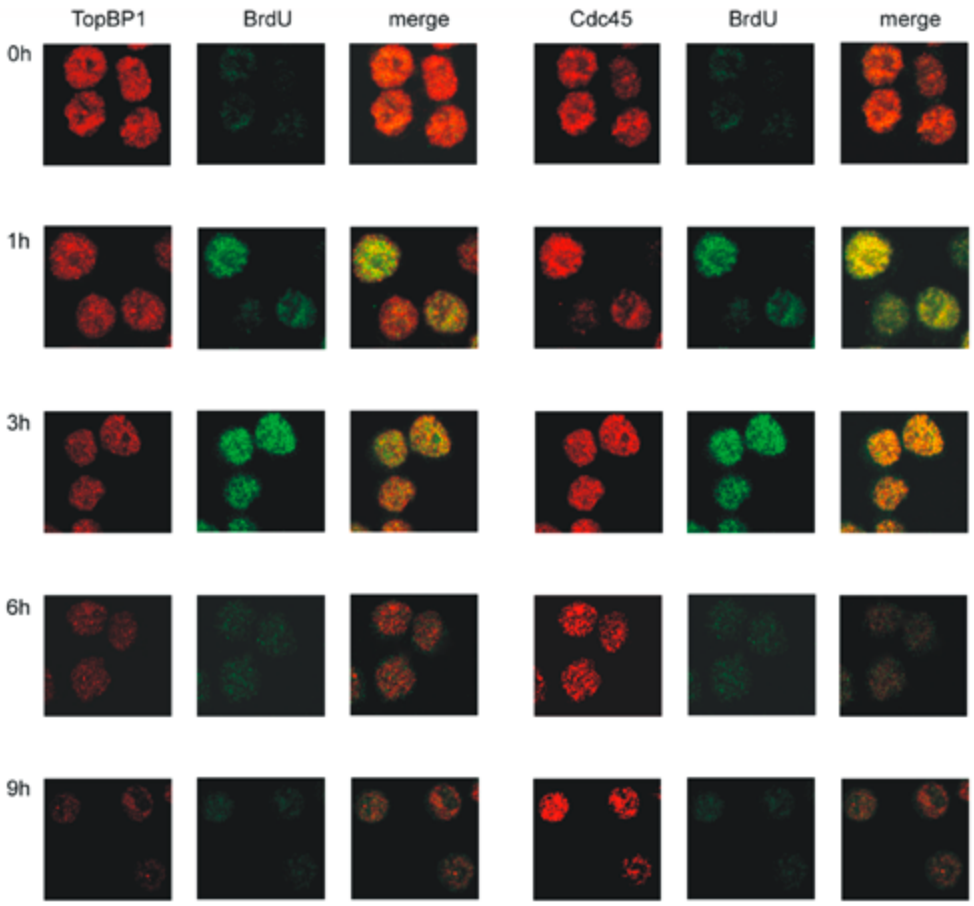


**Figure 2**
*In vivo* localization of TopBP1 and Cdc45 proteins in synchronized HeLa-S3-cells at different time points after release from the TdR-block. TopBP1 and Cdc45 co-localize with sites of BrdU incorporation. Synchronized HeLa-S3 cells were fixed and immunostained with specific antibodies against TopBP1, Cdc45 and BrdU, followed by rhodamine X-conjugated goat anti-rabbit IgG, Cy5-conjugated goat anit-rat IgG or fluorescein-conjugated goat anti-mouse IgG, respectively. Merge: yellow dots indicate co-localization of TopBP1 or Cdc45 on one hand and BrdU on the other hand. Support by G. D. Wieland (HKI Jena) is kindly acknowledged.

is involved in the formation of the initiation complex of replication in human cells and is required for the recruitment of Cdc45 to origins of DNA replication.

## 5 Host Cell Response to Pathogenic Infections

Group Leader: Thomas Munder, Hans Peter Saluz

### Cellular Stress

Here we have investigated host response mechanisms upon infection of cells by human pathogens. Transcriptomics and proteomics revealed essential genes and proteins which are involved in regulation of host cell response to etiologic agents. Within this framework, human fatty acid synthase was tagged as a major player in response to pathogenic infections.
In this study we have identified molecular targets of biologically active drugs involved in the infection process of pathogenic organisms. As a model system we have used the etiologic agent of human myocarditis, Coxsackievirus B3, because the interplay between host factors and virus components is hardly understood but crucial for the fate of the infected cells (Huber, 1997). Especially, host elements responsible for the changes observed during the course of CVB3-mediated myocarditis have not yet been investigated intensively (Henke et al.,2000; Henke et al., 2001). To rapidly expand the portrait of host gene expression involved in the pathogenesis of viral myocarditis and particularly to examine the expression of proteins, we used a proteome-wide approach. Proteins of infected and non-infected HeLa cells as well as HepG2 cells were separated on 2-dimensional gels and spots were analysed by peptide mass fingerprinting in combination with matrix assisted laser desorption/ionisation-mass spectrometry sequence analysis. Regulated proteins, e.g. nucleophosmin (nucleolar protein B23), lamin, the RNA-binding protein UNR

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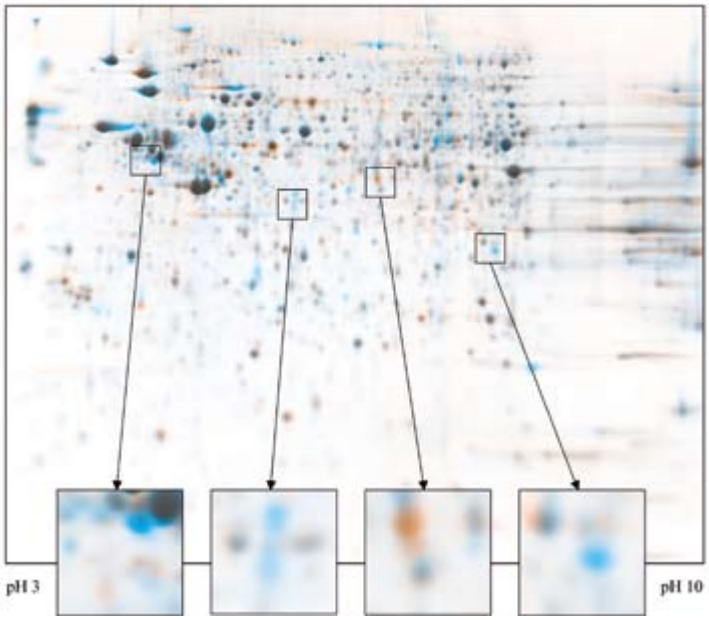


**Figure 3**
2-dimensional separation of the proteome of HepG2 cells infected with Coxsackievirus B3 and uninfected HepG2 cells.
Upregulated protein spots of infected cells appears blue whereas downregulated spots are orange.

or the p38 MAP kinase, respectively, were sorted according to their functional groups and interpreted in the context of the myocarditis process. Several proteins were found to be over expressed exclusively in infected cells. One of these proteins was identified as fatty acid synthase (FAS). To study the effect of FAS on the CVB3 infection process we inhibited the enzymatic activity by cerulenin and C75, two known highly specific and potent inhibitors. By this means the CVB3 replication was blocked significantly. These data showed that FAS is directly involved in the pathogenicity process and therefore suited as a therapeutic target in CVB3-induced diseases.

To investigate the nature of modified transcription in CVB3-infected human cells, DNA microarrays with an outstanding set of inflammatory related genes were used. Expression analysis of CVB3-infected HeLa and HepG2 cells revealed 34 genes with significantly altered mRNA expression levels upon infection Examples included genes of the TNF and NFB pathway. Therefore, both signaling routes may contribute to the CVB3 infection process.

Overall results demonstrated striking changes in protein/transcript composition between CVB3-infected cells and non-infected control cells. Different molecular mechanisms of host cells were influenced, including general stress-related events, cell signaling and transcription machinery.

To elucidate the pathogenicity process more in detail, interactions between CVB3 proteins and human host cell proteins were analysed. By this means, several cellular target proteins, e.g. telethonin, crystallin, titin, have been identified, which are localized in myocardial tissue and play an essential role in the integrity of the sarcomer. Thus, the virus may directly effect heart proteins by disturbing the sarcomeric structure, which may be a basis for virus-induced myocardial disease.

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## 6 Rapid Heat Block Thermocycling of Small Samples: Evaluation and Analysis of Fingerprinting Methodology

Group Leader: Alexander Tretiakov

### SpeedCyler

From its first-published account, polymerase chain reaction (PCR) has become a standard research tool in a wide range of laboratories and its enormous impact has been felt in basic molecular biology, clinical research, evolutionary studies and the various genome sequencing projects. Yet, the demands on PCR are still increasing and the need for rapid, inexpensive and efficient high-throughput thermocycling systems that can be easily coupled with post-PCR processing of multiple samples becomes more and more apparent. In keeping up with those demands we have developed a PCR machine, which is able to perform very rapid switches in heat block temperature for the parallel amplification of DNA, combined with a novel type of miniaturized ultrathin-walled microwell plates. Moreover, due to some specialities, such as a wine-press like heated-lid, small sample volumes (ranging from 500nl to 20µl) can be used, providing the conditions for highly efficient polymerase chain reactions. A number of up to 30 cycles can be completed within less than 15 minutes. What is more, the use of the ultra-thin walled microwell plates for rapid cycle DNA amplification has the great advantage of quick and easy delivering and also recovering of multiple microsamples. By this means, the overall throughput is highly increased compared to other existing "PCR" alternatives which involve conventional multiwell plates, glass capillary tubes or micro fabricated reactors.
The cycler and corresponding consumables have been commercialized by Analytic Jena AG and are commercially available.

During the past two years some companies started producing faster Peltier-driven heat-block thermocyclers able to complete standard 30-cycle PCR reactions in 30 minutes or less (Pray, 2005). Some of these machines are improved versions of standard heat-block thermocyclers, i.e. the Eppendorf Mastercycler ep and the Fast Cycler 9800 from Applied Biosystems. They use slightly modified thin-walled injection molded PCR tubes or multiwell plates. In addition, these two machines have slightly improved heating rates of 5-6°C per second and cooling rates of 4-4.5°C. However, some previous standard machines have average heating/cooling rates of 4°C per second and 3°C per second, respectively. These new instruments combine short two-step protocols and fast enzymes to complete the standard PCR reactions. However, they cannot be used for fingerprinting techniques because a classic three-step PCR is involved. Moreover, the slow Stoffel fragment of Taq polymerase has been shown to produce much more reproducible fingerprints than does standard Taq polymerase (Bassam et al., 1992).

In our study we used a novel Peltier-driven heat-block thermocycler, i.e. the SpeedCycler produced by Analytik Jena that differs from the above mentioned conventional two-cyclers in a number of improved characteristics. Ultrathin-walled microwell plates (20-40 microns) are specially optimized for small-volume samples, i.e. 0,5-15 µl, giving the cycler a much faster ramping rate, i.e. 10°C per second for heating and 6°C per second for cooling (Tretiakov and Saluz, US Pat 6,556,940). During a set of experiments using standard single-product reactions and three-step protocols, we obtained reproducible highly specific amplifications of 536 base-pair fragments of the human beta globine gene with KM29 and RS42 primers in 10-15 minutes. This corresponds to the results obtained by Wittwer and colleagues (1990) using a hot air glass capillary thermal cycler. According to the definition of rapid cycle PCR reactions (Wittwer and Garling, 1991), the SpeedCycler can be considered a "true" rapid cycle PCR machine, which is reported to be the world fastest heat-block thermocycler (Pray, 2005). At present we use it in order to test its performance for existing and novel fingerprinting techniques. First we tested the well-known, simple, and inexpensive reactions, i.e., RAPD and ISSR techniques (Awasthi et al.,2004). In addition, we worked on a new fingerprinting technique

called universally primed PCR (UP-PCR) that is becoming popular in research (Kang et al., 2002). This technique is similar to RAPD but uses longer primers, i.e., 20-nucleotides long, instead of 10-mer primers. The results are allegedly more reproducible due to higher annealing temperatures. The advantage of these techniques is that fingerprints can be resolved on simple agarose or acrylamide gels that do not need expensive sequencing equipment or large sequencing gels involving radioactivity necessary for high-multiplex fingerprinting reactions such as AFLP and the numerous variations thereof. Moreover, small acrylamide gels stained by SYBR Green were recently shown to resolve more polymorphic bands than large sequencing gels.

Our initial task was to reduce total amplification time. The techniques investigated took from 40 to 90 minutes to perform, depending on the polymerases used. The time required for rapid-RAPD was longer than for rapid-ISSR, and rapid-ISSR took longer than rapid UP-PCR. This is in accordance with the data obtained by the "second generation" cyclers and is explained by 1) RAPD and ISSR were subjected to the standard 45 cycles and 2) RAPD needs much lower annealing temperatures, i.e., 35° C. The commercially available Ready-to-Go RAPD Analysis Kit from Amersham optimized for conventional slow cyclers required more elongation time and usually resulted in amplification times for RAPD reactions of approximately 90 minutes. However, in conventional machines the reactions took approximately 4.5 hours using the company's protocol. Reducing the elongation time from 60 seconds to 30 seconds, the length of scorable fragments dropped to 400-600 base pairs. This can be explained by the slow speed and low concentration of the Stoffel fragment used in the kit. In contrast KlenThermN polymerase generates fingerprints covering from 150 to 1500 base pairs with elongation times of only 30 seconds in 40 minutes. Denaturation and annealing time usually did not last more than ten seconds. Standard Taq polymerases generally result in fingerprints with longer scorable fragments than those obtained by the Stoffel fragment but smaller than those obtained by KlenThermN polymerase. This demonstrates that the activity of the polymerases can be a limiting factor when the SpeedCycler is used to rapidly generate DNA fingerprints. However, even standard kits can produce more rapid results when the SpeedCycler is used (1.5 hours vs. 4.5 hours).

Any 2-primer fingerprinting technique can be converted to fluorescent versions by the same strategy, i.e., one primer is labeled and the second one isn't. This allows the use of denaturing sequencing gels that are less exposed to artifacts, e.g., heteroduplex products typical for non-denaturing gels. Components of different reactions were optimized according to standard practice. The products were separated on agarose or acrylamide mini gels. As all techniques require screening for informative primers, the overall time required for such screening reactions can be reduced. Theoretically, ISSR reactions can also involve an unlimited number of primers due to different micro satellite sequences or anchor nucleotides usually added to the 5'- or 3'-end of primers. UP-PCR uses long primers in pair wise combinations. Although the number of single primers may be small, the number of combinations of two primers may be very high, e.g., 20 primers may result in 210 different reactions. Moreover, when rapid reactions were performed, we observed that products separated on gels showed sharper bands compared to standard experiments. In addition, background fluorescence on gels was low. These features make analysis of fingerprints easier. Last, but not least, sample volumes were reduced to 10 µl to minimize reaction costs. An obvious additional advantage of using small volumes is the improved temperature homogeneity within the samples. Microwell plates compared to the above-mentioned rapid fingerprinting techniques (Tretiakov et al., 1994) have the following advantages: the reaction mixture is prepared directly in the wells without involving additional plastic consumables; the mixtures can be loaded by standard pipetting equipment; the microwell plates are rapidly sealed by standard sealing films, e.g., Microseal A Film, MJ Research; following PCR, samples are easily recovered

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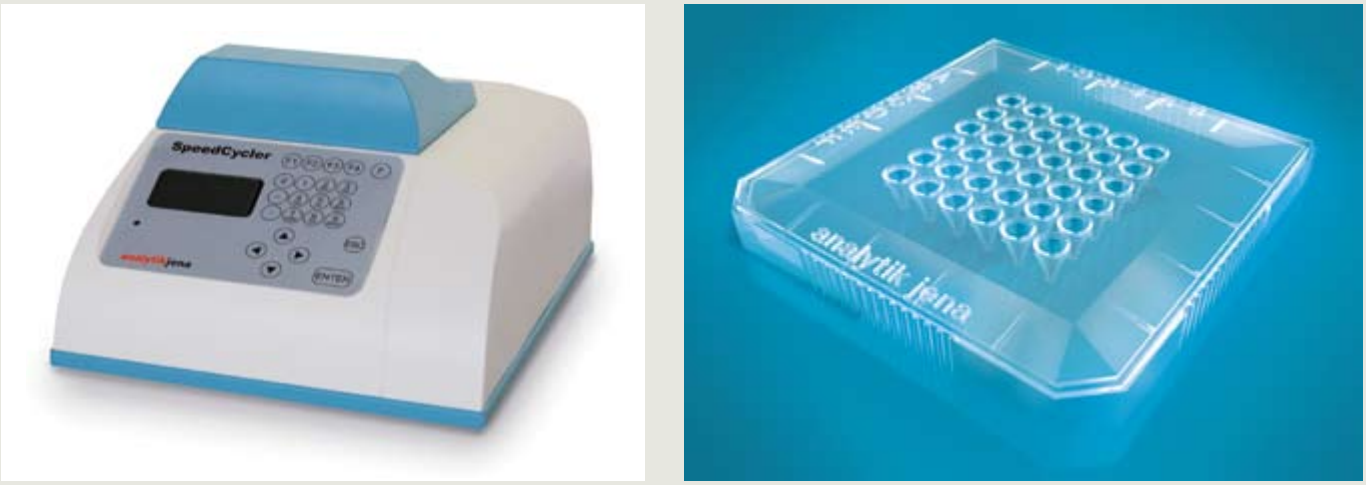


**Figure 4**
Rapid heat block thermocycler (SpeedCycler, Analytik Jena) and ultra-thin walled microwell plate.

by standard pipetting; all procedures correspond to internationally accepted practice for heat-block thermocycling. As the SpeedCycler is a Peltier-driven instrument that cools the block below ambient temperature it does not depend on laboratory air temperature, in contrast to air cyclers. For RAPD protocols which require samples to be cooled to 35°C and incubated, temperature can strongly influence final results.

## 7 Development of Rapid and Sensitive Methods for Pathogen Detection in Shrimp

Group Leader: Hans Peter Saluz

### SpeedCyler

Shrimp harvests from intensive aquaculture have recently declined in areas which have been productive for many years. Because the most convenient explanation for these crop failures has been the occurrence of infectious diseases, there is a need to consider shrimp health from a holistic point of view. For effective shrimp health maintenance and surveillance, the following components need consideration: development of rapid and sensitive methods for pathogen detection; establishment of shrimp tissue cultures for virology; immunological studies, toxicological studies and drug efficacy evaluation. Within the frame of this application we plan to work on the development of a novel, very rapid and highly sensitive technology for detection and monitoring of the major pathogens in parallel. A great advantage to undertake such a project concerns a novel rapid PCR technology invented, developed and patented in our laboratory. By this unique means we are able to develop low-cost and highly efficient assays to reach our goals, which are of great scientific and economic interest for our indonesian partners.

The major aims of this application concern the development of a model approach concerning a novel, very rapid and highly sensitive technology for detection and monitoring of the major shrimp pathogens in parallel.

It is important for research to come up with new means to secure the stability, sustainability and profitability of the shrimp industry. Within this frame, a great problem concerns infectious and non-infectious shrimp diseases, which continuously plagued the various sectors of the industry.
Diseases caused by bacteria, fungi, protistans and viruses are considered very significant to shrimp culture. Bacteria are considered the most economically significant disease agents of shrimp. In addition, infections by fungi result in heavy mortalities in larval stocks. At least 15 viruses are known to infect cultured and wild marine penaeid shrimp.
Therefore, we would like to establish a novel model approach to analyse in parallel the above pathogens in very rapid means and involving tiny amounts of biological material. This model approach will be used to establish generally applicable shrimp diagnostics involving all major shrimp pathogens thus allowing a rapid interference with adequate drugs, etc.

The use of new gene probe technologies that rely on demonstrating specific nucleic acid sequences offers an opportunity to detect and monitor shrimp pathogens at much earlier stages of infection. Therefore, we aim to develop a novel, very rapid and highly sensitive technology for detection and monitoring of the major pathogens in parallel. Upon designing and synthesizing oligonucleotide primers which are unique for each pathogen and/or entire groups of pathogens, we will make use of a novel rapid PCR technology where minute amounts of diseased shrimps, e.g. a small part of leg, in combination with the above primers and special labelling procedures will be used for diagnostics. The great advantage of this technology will be the extremely sensitive parallel analysis of many pathogens and host at once. Commercially available rapid PCR-systems (LightCycler from Roche) are based on capillaries and therefore are not suitable for automized high parallel investigations. So far, there is no comparable complete system available, thus fulfilling the aims of many investors.

At present, the main goal of the shrimp industry is to meet the growing demand in an optimum manner without damaging the environment. The role that shrimp disease research must play to attain such a goal must be seriously considered by both the private and government sector, and by national and international organizations.

It is obvious that collaborative effort involving all sectors is needed to attain sustainability. However, a sustainable industry can only be achieved if considerable investment is provided. In addition, the planned approach could be expanded, thus involving Germany biotechnologically as provider of relevant technology. Finally, such a project might strengthen also certain political relations between Germany and Indonesia.

## 8 Fundamentals of DNA-Chip/Array Technology for Comparative Gene-Expression Analysis

Group Leader: Hans Peter Saluz

### Chip/Array Technology

One of our objectives has been the identification of differentially expressed genes in various cell lines and/or tissues upon microbial infection. To that end we have set out to adopt and/or develop microarray sytems, which allow the simultaneous handling of thousands of samples within sets of different biomolecules under nearly identical experimental conditions. Comparative expression profiling is one of the remarkable things about DNA chips and now every one is finding new ways to make them. From the results so far, it seems that DNA-chip technology becomes as powerful as PCR within the next few years. A clear trend of transition has started in expression-profiling studies.

The real challenge in expression arrays is in developing the experimental design to exploit the full power of global perspective experimental manipulation, like responses to the microenvironment and state of the arrays, hybridization time, scanning procedures and other related aspects which need to be rigorously controlled. Another challenge of concern is the study of the expression levels of very small quantities of target tissues. One should keep in mind while interpreting the data from DNA chips that transcription levels cannot be equaled to protein abundance or the rate of transcription is dependent on the half-life and decay direction of the mRNA. Also the signal strength does not reflect the level of potential translating mRNA molecules as the protein can be regulated posttranslationally. Although the problem is a pleasant one, it can be improved and it will provide us the great insight into the cellular function and will allow us to come closer to the art of the network functions in the cell.

In our laboratory fabrication of cDNA microarrays has become an important tool to unravel basic aspects concerning host-pathogen interactions. Within the framework of microarray technology we have established a human stress-related cDNA library of several thousands of cDNA clones. All these clones (originally 75.000 cDNA clones) were characterized by an extensive analysis. By this means we were capable to subdivide transcripts for many pathology groups. All transcripts were identified and verified. Furthermore, all clones containing contaminating repetitive elements were excluded and all EST transcripts were separated. Last but not least several thousand novel potential drug targets could be identified.

In addition, we have been interested in the fabrication and evaluation of sequence-specific oligonucleotide miniarrays for molecular genotyping of microbials. Accurate genotyping not only requires discrimination between low- and high-risk pathogens for effective diagnosis or disease management, but also requires the identity of the specific strain or type of the microbe involved in pathogenesis. The majority of these assays require DNA amplification followed by genome identification either through sequencing or hybridization to specific oligonucleotide probes. We evaluated the use of DNA microchip assays as a simple and easy-to-use procedure for genotyping. Various methodological parameters were optimized for single-base mismatch discrimination on a DNA microarray. The fabrication procedures involved substrate chemistry for immobilization. The effect of various buffers and features associated with oligonucleotide sequences were standardized. The assay was evaluated on a low-density genotyping chip containing the sequences of various Human Papilloma Virus (HPV) subtypes.

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Bundesministerium für Bildung und Forschung
Forschungsverbund mit Indonesien: SPICE – Schnelle Diagnose und Überwachung von Shrimpkrankheiten und Shrimppathogenen in natürlicher Umgebung und in der Aquakultur
Hans Peter Saluz

Bundesministerium für Bildung und Forschung
Eine neuartige parallele und sehr schnelle Technologie zum Nachweis und zum Monitoren von Shrimp-Krankheiten
Hans Peter Saluz

Industry
IPHT e.V., Jena
Einzel-Zell-Identifikation und Einzel-Zell-Gen-Expressionsdiagnostik durch in-situ RT-PCR in Mikrodurchflußreaktoren
Hans Peter Saluz

Industry
GMBU e.V., Jena
Entwicklung von Biochips von DNA-Modellchips für einen Spectral-Imaging-Sensor
Hans Peter Saluz

## Selected publications
(HKI authors in bold)


**Goellner S**, Schubert E, Liebler-Tenorio E, Hotzel H, **Saluz HP**, Sachse K (2006) Transcriptional response patterns of *Chlamydophila psittaci* in different in vitro models of persistent infection. *Infect Immun* 74, 4801-4808.

**Goellner S**, Steinbach D, Schenk T, Gruhn B, Zintl F, Ramsey E, **Saluz HP** (2006) Childhood acute myelogenous leukaemia: Association between PRAME, apoptosis- and MDR-related gene expression. *Eur J Cancer* 42, 2807-2814.

**Krügel H**, Becker A, Polten A, Grecksch G, Singh R, Berg A, Seidenbecher C, **Saluz HP** (2006) Transcriptional response to the neuroleptic-like compound Ampullosporin A in the rat ketamine model. *J Neurochem* 97, 74-81.

**Nestler M**, Martin U, **Hortschansky P**, **Saluz HP**, Henke A, **Munder T** (2006) The zinc containing pro-apoptotic protein siva interacts with the peroxisomal membrane protein pmp22. *Mol Cell Biochem* 287, 147-155.

**Usskilat C**, **Skerka C**, **Saluz HP**, **Hänel F** (2006) The transcription factor Egr-1 is a regulator of the human TopBP1 gene. *Gene* 8, 144-150.

**Wollmann Y**, **Schmidt U**, **Wieland GD**, **Zipfel PF**, **Saluz HP**, **Hänel F** (2007) The DNA topoisomerase IIbeta binding protein 1 (TopBP1) interacts with poly (ADP-ribose) polymerase (PARP-1). *J Cell Biochem* 102. 171-182.

**Munder T**, Henke A, Martin U, **Saluz HP** (2007) Apoptotic processes during coxsackievirus-induced diseases. *Trends in Cell & Molecular Biology* 1, 93-101.

Schenk T, Stengel S, Steinbach D, **Saluz HP** (2007) Hypomethylation of PRAME is responsible for its aberrant overexpression in human malignancies. *Genes Chromosomes Cancer* 46, 796-804.

**Schmidt U**, **Wollmann Y**, **Franke C**, Grosse F, **Saluz HP**, **Hänel F** (2008) Characterization of the interaction between the human DNA topoisomerase IIbeta-binding protein 1 (TopBP1) and the cell division cycle 45 (Cdc45) protein. *Biochem J* in press.

**Wollmann Y**, **Schmidt U**, **Wieland GD**, **Zipfel PF**, **Saluz HP**, **Hänel F** (2007) The DNA topoisomerase IIbeta binding protein 1 (TopBP1) interacts with poly (ADP-ribose) polymerase (PARP-1) *J Cell Biochem*. 102, 171-182.


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# Department of Infection Biology

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The department of Infection Biology focuses on the role of the complement system for immune evasion of pathogenic microbes, and on immune dysfunction in form of autoimmune diseases. Pathogenic microbes evade host immune attack by expressing surface proteins that act as receptors for soluble host innate immune regulators. The identification and characterization of such microbial surface proteins in particular of fungal origin, reveals a general immune evasion mechanism and identifies novel virulence factors. The same soluble host immune regulators, which are utilized by pathogens, play a protective role in the human host and are essential for maintaining tissue integrity. Mutations of such regulators, like Factor H result in immundysfunction and autoimmune kidney diseases, such as membranoproliferative glomerulonephritis (MPGN), hemolytic uremic syndrome (HUS) and specific sequence variations increase the risk for the common retinal disease age related macular degeneration of the eye (AMD).

## INTRODUCTION | EINLEITUNG

**Head:**
**Prof. Dr. Peter F. Zipfel**

Die Abteilung Infektionsbiologie beschäftigt sich mit der Rolle des Komplementsystems bei der Immunevasion von pathogenen Mikroorganismen und der Immundysfunktion bei Autoimmunerkrankungen. Pathogene Mikroorganismen exprimieren Oberflächenproteine, die als Rezeptoren für lösliche Immun- und Komplementregulatoren des Wirtes dienen und die eine wichtige Funktion bei der Immunevasion spielen. Die Identifizierung und funktionelle Charakterisierung dieser mikrobiellen Proteine zeigt einen generellen Mechanismus der Immunevasion und führt zur Identifizierung von neuen Virulenzfaktoren, welche für das Überleben des Pathogens im Menschen als immunkompetenten Wirt entscheidend sind. Die von pathogenen Mikroorganismen genutzten Komplementregulatoren des Wirtes spielen eine zentrale Rolle bei der Aufrechterhaltung der Gewebsintegrität des Menschen. Mutationen in diesen Regulatorproteinen führen zu Immundysfunktion und Autoimmunerkrankungen der Niere, wie Membranoproliferativer Glomerulonephritis, der atypischen Form des Hämolytisch Urämischen Syndroms und definierte Sequenzvariationen einzelner Gene erhöhen das Risiko für die altersabhängige Makuladegeneration des Auges (AMD).

## 1 Innate Immunity: The Role of Complement for Fungal Evasion

Group Leader: Peter F. Zipfel

### Immune Evasion Mechanisms of the Human Pathogenic Yeast *Candida albicans*

The complement system provides the first defense line of innate immunity and is an immediately acting defense system of the vertebrate host. Infectious agents, which invade the human host are attacked and eliminated by the activated complement system, in contrast pathogens survive complement attack as they inhibit complement activation directly at their surface. Pathogenic fungi, such as *Candida albicans* and *Aspergillus fumigatus*, acquire soluble human complement regulators to their surface and utilize these surface attached host regulators for immune evasion. We have identified several fungal CRAS-Proteins (complement regulator acquiring surface protein) which have been cloned and recombinantly expressed. Antisera raised against the recombinant proteins demonstrate expression of these proteins on the yeast surface. Apparently these *Candida* proteins are multi functional, as they bind several host plasma proteins, like the complement regulators Factor H, FHL-1 an additional member of the Factor H protein family and plasminogen. The bound host proteins maintain regulatory activity, i.e. surface attached Factor H and FHL-1 control complement activation and attached plasmin(ogen) shows proteolytic activity. In addition human fungal pathogens utilize a second layer of immune defence as they secrete proteases which degrade host proteins. The proteases have been identified as SAP1 and SAP3, which are secreted by both yeast and hyphal forms of *C. albicans*. Multiple immune effector proteins act at the interface of the pathogen and the human host. The molecular characterization of these proteins and immune evasion strategies identifies relevant virulence factors, which represent novel targets to interfere directly with infection.

*Candida albicans* and also other human pathogenic fungi evade the host complement system by binding human plasma and immune regulators to their surface. *C. albicans*, *Aspergillus fumigatus* and also the human dermatophyte *Arthroderma benhamiae* bind the human alternative pathway regulators Factor H, FHL-1 and CFHR1, and also the classical pathway regulator C4 binding protein (C4BP) as well as plasminogen to their surface (Fig. 1 and 2). It is the interest of our group to identify and characterize these fungal surface proteins and to characterize the role of these yeast proteins for immune evasion.

Several *C. albicans* proteins that bind the human alternative pathway regulators Factor H and FHL-1, as well as C4BP were cloned and identified. A total of seven Factor H and FHL-1 candidate binding proteins of *C. albicans* were isolated. These candida proteins which are likely virulence factors are termed *Candida albicans* **c**omplement **r**egulator-**a**cquiring **s**urface **p**roteins (CaCRASP-1 to CaCRASP-7). The corresponding genes of the seven molecules were amplified from *C. albicans* genome, cloned and recombinantly expressed in the *Pichia pastoris* system. The CaCRASP-1 was recently identified as a surface-exposed plasminogen-binding protein of *C. albicans*. Following recombinant expression the 33 kDa CRASP-1 was purified to homogeneity and used for binding assays.

Three human plasma proteins, the two immune regulators Factor H and FHL-1, as well as plasminogen bind specifically to the candida CRASP-1 and FHL1 and plasminogen bind with similar intensities. When bound to *Candida* CRASP-1 the host complement regulators maintain regulatory activities. Attached to

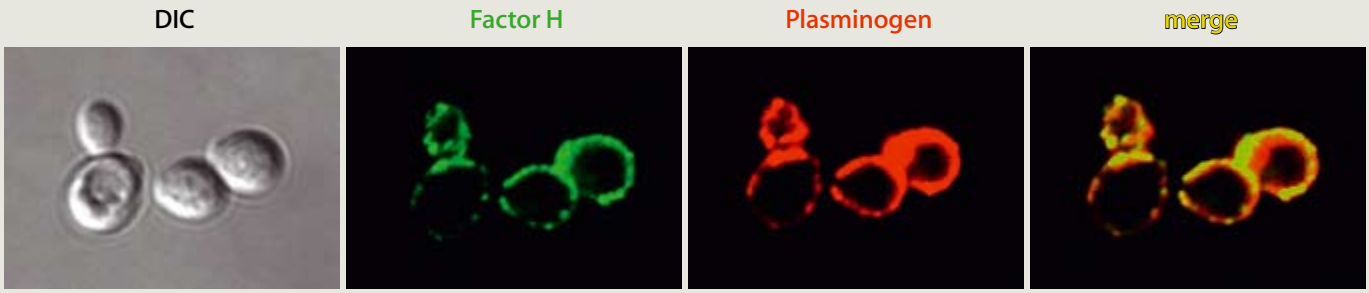
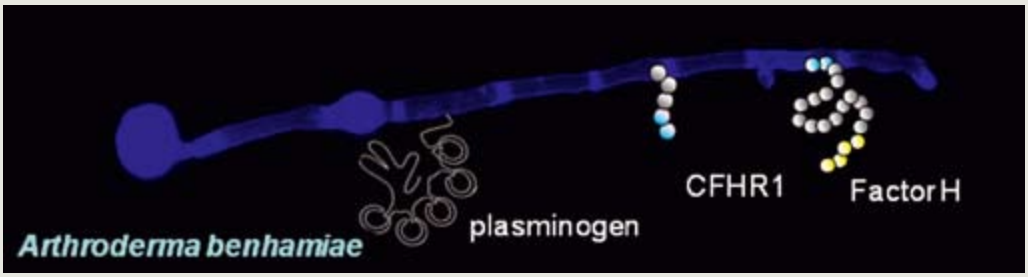



**Figure 1**
**Binding of Complement Factor H and Plasminogen on the surface of the human pathogenic yeast *C. albicans*.**
*Candida* cells were incubated in human serum and surface bound host plasma proteins were identified by immunostaining. The confocal images reveal simultaneous binding of Factor H and Plasminogen to the same ligands, indicated by the yellow staining in the overlay of the two images (merge).

**Figure 2**
**The human dermatophyte *A. benhamiae* binds host immune regulators.**
*A. benhamiae* shown by the blue color (Dapi staining) when incubated in human serum binds several host plasma proteins in form of Plasminogen, the complement Factor H related protein 1 (CFHR1) and the central complement inhibitor Factor H.




CRASP-1 Factor H and FHL-1 inhibit complement activation and block the generation of the complement activation product C3b. Thus the acquired host regulators mediate complement control at the surface of the yeast cell. Similarly plasminogen bound to immobilized CRASP-1 is converted to plasmin by the proteases uPA and tPA, and the newly generated plasmin shows proteolytic activity and cleaves the chromogenic substrate S2251 (Fig. 3).

In addition to complement control, the surface attached complement regulators Factor H and FHL-1 enhance interaction of *Candida* cells and hyphae to host epithelial and endothelial cells. When bound to the surface of *Candida* both human plasma proteins enhance attachment to host cells in a role similar to fibronectin and fibrinogen. Similarly the role of attached plasmin for interaction as well as degradation of the extracellular matrix is a relevant point to study.

Immune evasion of the human pathogenic yeast *Aspergillus fumigatus* and of the human dermatophyte *A. benhamiae* is studied in collaboration with Professor A. Brakhage, Department of Molecular and Applied Microbiology. So far binding of the host immune regulators Factor H, FHL-1 and CFHR1 was identified and in addition binding of plasminogen to the surface of both *A. fumigatus* and *A. benhamiae* was shown. Bound proteins are functionally active and thus mediate immune response and proteolytic activity.

A second form of immune defense of pathogenic yeast was identified for *C. albicans, A. fumigatus* and also *Arthroderma benhamiae*. These pathogenic fungi secrete proteases that act on central host complement proteins. Induction of these proteases was observed upon hyphal growth for *C. albicans*, and specific protease inhibitors were used for the characterization of these proteases. In collaboration with Prof. B. Hube, Department of Microbial

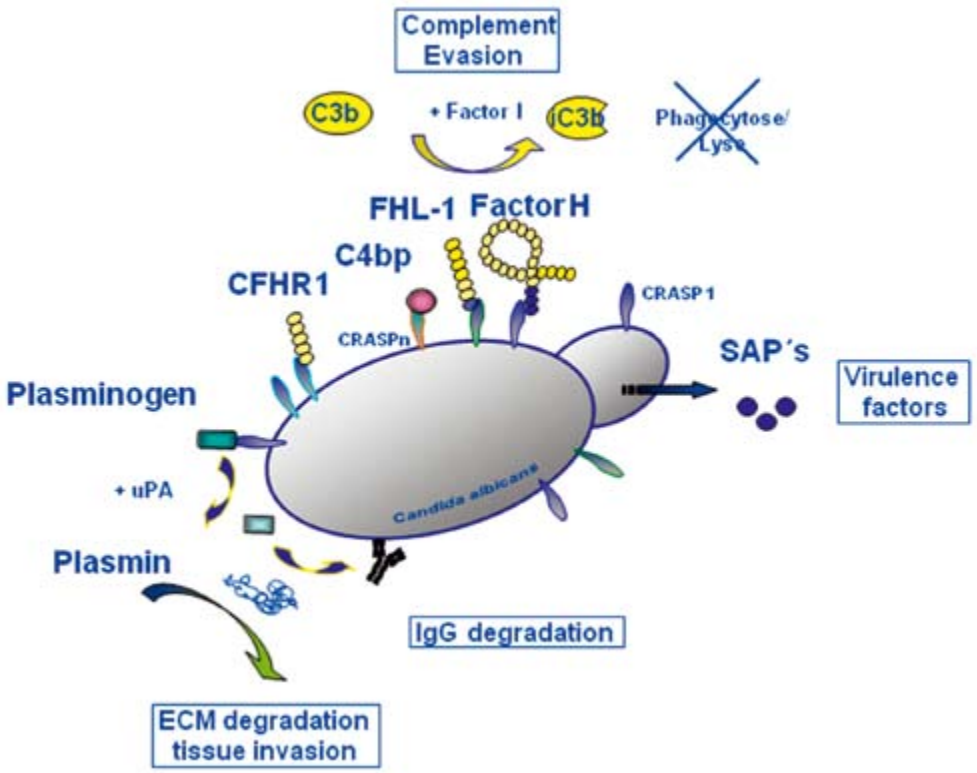



**Figure 3**
**Immune evasion strategies of human pathogens.**
Human pathogens as depicted here for the human pathogenic fungus *C. albicans* bind multiple host immune regulators of the complement and of coagulation system and in addition secrete proteases. The acquired host effector proteins aid in complement evasion, ECM degradation and tissue invasion, as well as IgG degradation. Pathogen encoded secreted aspartyl proteases (SAPs) act as virulence factors and also degrade host immune effector proteins.

Pathogenicity Mechanisms, the secreted Aspartyl Proteases SAP1, SAP2 and SAP3 were identified as relevant molecules.

Thus pathogenic fungi utilize multiple mechanisms to inactivate host complement. The results allow to decipher the sequential response of fungi upon infection of the human host. During the immediate, initial phase of infection, acquisition of host complement regulators allows complement control directly at the surface of the pathogen. Following this first step, the pathogen synthesizes proteases that further cleave host complement products, and that may initiate the next steps that are important to establish an infection, i.e. degradation of extracellular matrices and tissue invasion.

## 2 Innate Immunity: The Role of Complement for Microbial Immune Evasion

Group Leaders: Christine Skerka, Peter F. Zipfel

### Immune Evasion Mechanisms of the Human Pathogenic Microbes

The complement system provides the first defense line against invading microbes and is a central and immediately acting immune system of the vertebrate host. Infectious microbes which invade the human host are attacked and eliminated by the activated complement system, but pathogenic microbes control complement activation at their surface and survive. A wide range of pathogens, including Gram-negative and Gram-positive bacteria, multicellular parasites, as well as viruses acquire soluble human complement regulators to their surface. Regulators such as Factor H, members of the Factor H protein family, like FHL-1 and CFHR1

or the classical pathway regulator C4BP are bound to the surface. Several of these microbial surface proteins have been identified and cloned from Gram-negative bacteria, including *Borrelia burgdorferii* and *Pseudomonas aeruginosa*, as well as the Gram-positive bacterium *Streptococcus pneumoniae*. Binding of host immune regulators is a general and conserved mechanism and based on these common features. These microbial proteins are termed CRASP (i.e. Complement Regulator Acquiring Surface Proteins). At present we are characterizing five CRASPs from *Borrelia burgdorferii*, two CRASPs from *Pseudomonas aeruginosa*, one CRASP from *S. pneumoniae* and the Sbi protein from *Staphylococcus aureus*. The characterization of such surface proteins, which are derived from a wide range of pathogenic microbes, allows to define the general immune evasion strategies and the identification of the common features. The various surface proteins, which are derived from distinct microbes show related or even identical function i.e. binding of host plasma proteins but they lack any direct sequence similarity. The aim of this project is the characterization of the common structural and functional features of these diverse microbial proteins.

Human pathogenic microbes express specific surface proteins which bind host complement and immune regulators. It is the interest of our group to identify such surface proteins and to characterize their role in immune and complement evasion. An emerging feature is that these pathogenic surface proteins bind multiple ligands and several host proteins such as complement regulators and effector molecules of the coagulation cascade. These complement escape proteins show a high degree of sequence and antigenic diversity between individual strains and isolates. However, at the same time these complement evasion proteins display conserved binding characteristics for the various host immune regulators.

The Gram-negative bacterium *B. burgdorferi* expresses a total of five CRASPs at the surface and Factor H and FHL1 binding correlates directly with serum resistance. Individual isolates express either all five CRASPs or express different combinations of these proteins. The various borrelia CRASPs show unique, as well as common binding characteristics. CRASP-1 and CRASP-2 bind Factor H and FHL-1, but not CFHR1. In contrast CRASP-3, CRASP-4 and CRASP-5 bind Factor H and CFHR1, but not FHL-1. These binding specificities suggest a unique role for each individual CRASP in the immune response of *B. burgdorferii*.

A detailed binding analysis shows that the host regulator Factor H has a two point interaction and FHL-1 a one point interaction with BbCRASP1. Factor H binds to CRASP1 and CRASP2 with domain SCR 7 and with the C-terminal surface recognition domain. Consequently in its bound conformation the complement regulatory region, located in the N-terminal four domains is accessible, mobile and functionally active. By expressing multiple, distinct surface proteins with unique binding characteristics, *Borrelia* display several interaction sites for the host regulators. Thus by increasing the number of attachment points, such a surface decoration with host proteins allows flexibility and provides various possible interaction sites.

Particularly the serum resistant forms of the pathogenic bacterium *Pseudomonas aeruginosa* bind the host immune regulators Factor H and CFHR1. The immune evasion strategy of this Gram-negative bacterium is different from that of *Borrelia*, as this pathogen utilizes two separate strategies to control complement activation at its surface. The pathogenic forms express endogenous surface proteins that inhibit complement activation and inactivate C3b which is generated on the bacterial surface. In addition *P. aeruginosa* expresses surface proteins that act as receptors for host immune regulators and that bind complement Factor H and CFHR1, an additional member of the Factor H protein family.

Serum resistant Gram-positive *S. pneumoniae* express surface proteins that bind Factor H and FHL1. The two CRASPs, which are also termed PspC and HIC were utilized to characterize the binding and interaction between the bacterial and the host proteins. Apparently the two host

proteins attach with two domains to the bacterial proteins, thus forming a two point interaction. In addition the immune effector proteins of *S. pneumoniae* utilize surface bound Factor H as a bridging molecule which mediates attachement to host cells via specific immune receptors.

In summary, evasion of complement attack is a common feature of pathogenic microbes. All analyzed pathogens utilize functionally related surface proteins, that bind host plasma proteins and the complement regulators Factor H, FHL1 and CFHR1. The detailed characterization on the molecular level identifies common binding characteristics and general immune evasion strategies of these microbes. Common features of this diverse group of CRASPs reveals, that:

(i) pathogenic microbes utilize several, different surface proteins for immune evasion,
(ii) the individual microbial proteins bind multiple host plasma proteins,
(iii) individual CRASPs are highly polymorphic and show a significant sequence variability between different strains,
(iv) attached host regulators are functionally active and attached to the microbe they inhibit C3b formation.

The various CRASPs have the identical function as they bind the same host proteins. However so far any detailed sequence comparison of the individual proteins and of the localized interaction domains showed diverse sequence motives and no conserved pattern. However as the diverse microbial proteins attach the host regulators at the same sites and domains it will be of great interest to identify additional features which are common for this type of interaction.

A novel feature is binding of the complement Factor H related protein CFHR1 to pathogenic microbes. Binding has been demonstrated for several pathogens including CRASP3 – CRASP4 of *B. burgdorferi*, Tuf of *P. aeruginosa*, Scl surface protein of *S. pyogenes*, and CRASP2 of fungal pathogens. Apparently CFHR1 competes with Factor H for binding to these microbial surface proteins and thus has an indirect inhibitory effect on Factor H function. However the exact role of CFHR1 is pending. As surface attached CFHR1 is beneficial for the pathogen it will be highly interesting to identify the exact function of CFHR1, which is a challenge for our future work.

## 3 Innate Immunity: Complement Dysfunction in Human Diseases

Group Leaders: Christine Skerka, Peter F. Zipfel

Defective complement regulation results in human auto-immune diseases. Complement is an aggressive host immune defense system that is aimed to attack invading microbes. Host cells need tight control of their surfaces to prevent tissue injury. Defective control results in different diseases, like severe kidney diseases, such as the atypical form of Hemolytic Uremic Syndrome (HUS) and Membranoproliferative Glomerulonephritis type II (MPGN II). In addition, the retinal disease age related macular degeneration (AMD), which is the major cause of blindness in elderly people is also associated with variations in human complement regulators. Homozygous or compound heterozygous mutations of the human complement regulators like Factor H result in MPGN, whereas heterozygous mutations result in HUS. In addition, deletion of a chromosomal fragment within the Factor H gene cluster forms a risk for HUS however has an opposite protective effect in AMD. The goal of this project is to understand and describe how mutations in certain complement regulators, such as single amino acid exchanges affect protein functions and how deletion of the CFHR1 and CFHR3 genes predispose to disease. The identification of the functional consequences of mutated, truncated or deleted Factor H proteins derived from MPGN, HUS or AMD patients will likely demonstrate that local complement control is important for tissue integrity, especially under infection processes.

Defective complement control causes **atypical hemolytic uremic syndrome** (aHUS). Mutations in complement regulators, particular as

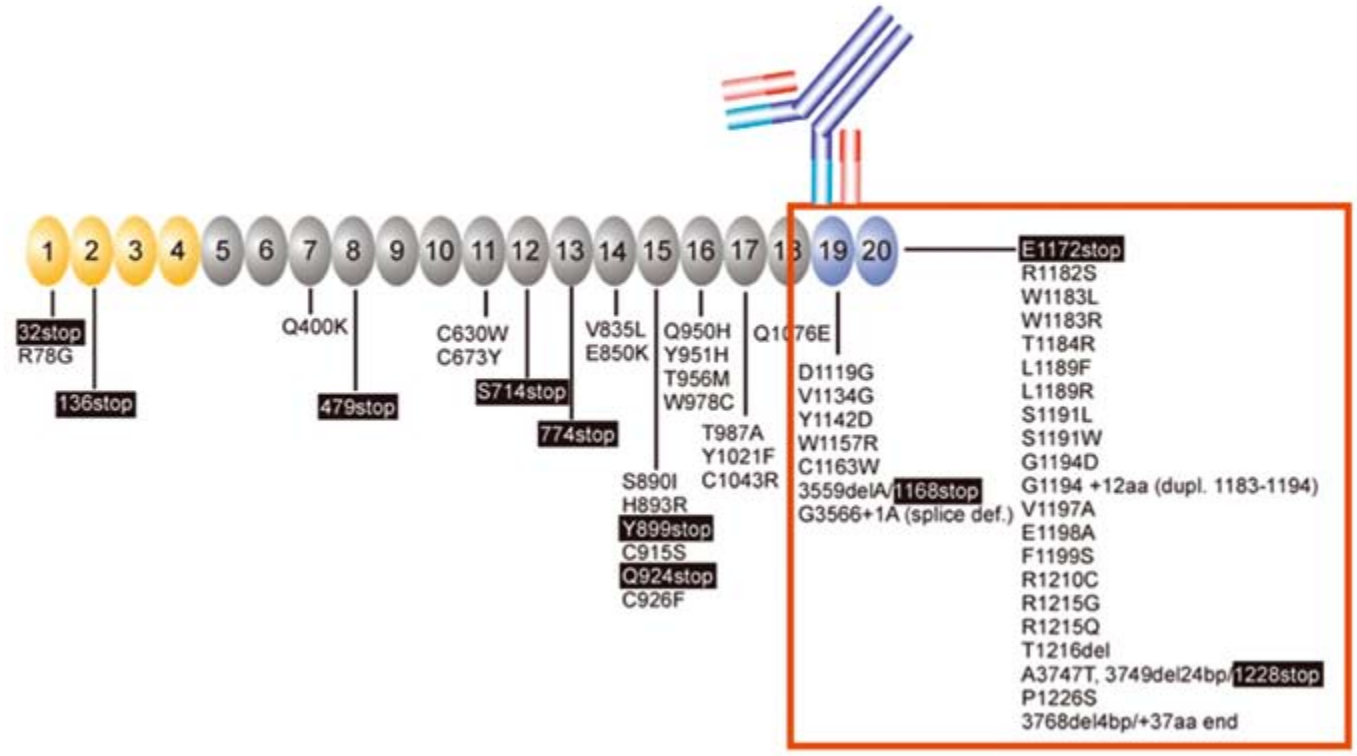



**Figure 4**
**Factor H gene mutations in the human kidney disease hemolytic uremic syndrome (HUS).**
Mutations in the Factor H gene are associated with HUS. The identified mutations in the Factor H gene cluster in the C-terminal surface binding region of the protein in domains SCRs 19 and 20. The vast majority of the mutations occur in a heterozygous setting. Thus the patients have one defective and one intact allele.

Factor H were reported in several aHUS patients (Fig. 4). The majority of Factor H mutations cluster in the C-terminal surface binding region of the protein and result in reduced surface and cell binding. The role of Factor H mutations in aHUS is demonstrated in a cellular model system that allows to study the action of Factor H at the surface of endothelial cells. Factor H protects the surface of human umbilical vein endothelial cells (HUVEC) and this protective activity is evidenced by the inactivation of surface deposited C3b, inhibition of MAC formation and reduced cell lysis (Fig. 5).

This defect has been demonstrated for a mutant Factor H protein derived from a patient who is heterozygous for a Factor H gene mutation at position 3587. This nucleotide exchange introduces a premature stop at amino acid 1172, within the most C-terminal domain in SCR 20. The mutant protein is expressed in plasma and identified following SDS-PAGE separation as a truncated protein with increased mobility. The mutant Factor H protein was purified to homogeneity from plasma of the patient and used for functional analyses. In the endothelial cell model the truncated mutant Factor H showed severely reduced cell-binding activity, which resulted in stronger complement activation at the surface of endothelial cells. The relevance of mutations in the C-terminus of Factor H is underlined by functional assays with additional recombinant proteins in which disease associated mutations were introduced by *in vitro* mutagenesis. These analyses show that single amino acid exchanges in the central surface and cell binding region of Factor H affect the important regulatory functions on the cell surfaces.

The characterization of Factor H functions regarding protection of self cells and the integrity of homeostasis was also shown in experiments using monoclonal antibodies (mAB) which bind to defined domains in Factor H.

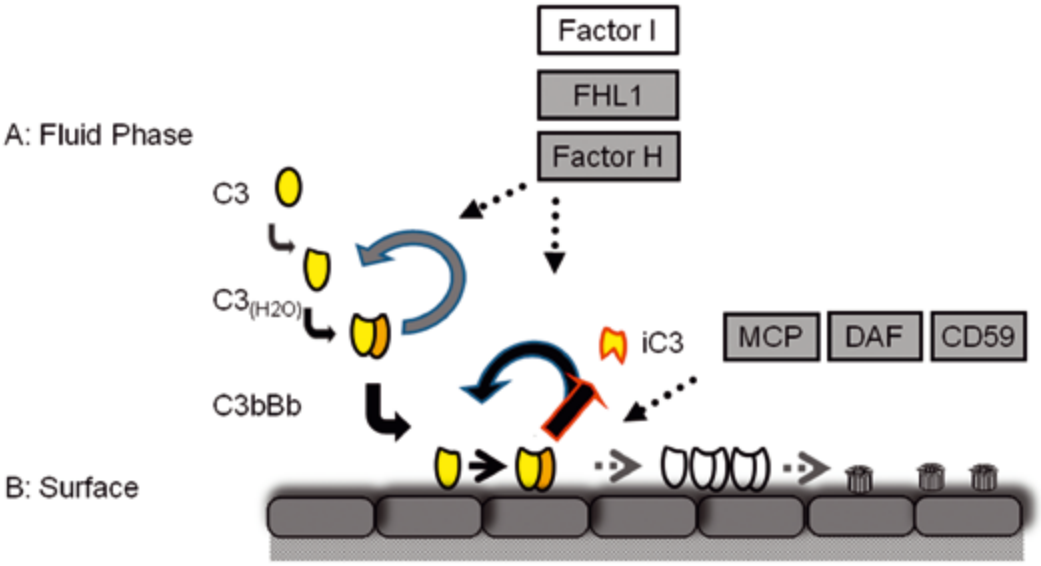



**Figure 5**
**Defective Regulation of the Alternative Complement Pathway as the Basis for Pathophysiology of Complement Associated Kidney Diseases and AMD.**
Continuous activation of the alternative complement can result in damage of biological surfaces. Activation of the alternative pathway occurs spontaneously, in the fluid phase, in plasma and is initiated by default. The activation generates an active C3 molecule (C3H2O) which binds Factor B and consequently forms the first fluid phase convertase C3(H2O)Bb. This enzyme generates more C3b, which can deposit on nearby surfaces and generate – by the same mechanism – a surface bond convertase. The initial reactions are rather slow and allow control and inhibition. In its default setting activation proceeds, amplifies and translates from the fluid phase onto nearby surfaces. Following a powerful amplification reaction this activation results in opsonization of the surface with C3b molecules or formation of the membrane attack complex (MAC) which generates pores in the membrane. The default scenario is favorably on foreign surfaces such as microbes and results in their elimination. However on self surfaces the same activation is deleterious and causes self damage. Consequently cell and tissue surfaces utilize a set of membrane bound and surface acquired regulators that inactivate and activate the cascade. Apparently several regulators in form of surface bound (MCP/CD46 and DAF/CD55) together with soluble regulators (Factor H, FHL1 and the serine protease Factor I) mediate complement control, they inactivate C3b and dissociate the existing C3bBb convertase. This inactivation is essential for maintenance of surface integrity. Additional scenarios which affect the activity of this central amplification convertase include in HUS the presence of autoantibodies which bind and block the binding functions of Factor H. In MPGN two types of autoantibodies are reported: the less frequent variant a lambda light chain dimer binds to and blocks the regulatory function of Factor H and the more frequent variant C3NcF, which binds to and stabilizes the newly formed amplification convertase C3bBb positioned in the outside region of the CCP domain.

Blocking the C-terminus of Factor H resulted in enhanced complement activation at the cell surface. Such C-terminally binding mABs which affect surface binding of Factor H are of special interest, as autoantibody directed against the same protein domain were identified in a subgroup of aHUS patients. In the Jena HUS cohort of 147 HUS patients, 16 patients with autoantibodies were identified. All autoantibodies bind to the C-terminus of Factor H and interfere with surface attachment of Factor H on cells and biomembranes. This effect explains that Factor H abnormalities, in form of C-terminal mutations and the presence of autoantibodies may result - upon triggering of the complement cascade - in endothelial damage and progression to aHUS.

Interestingly the subgroup of HUS patients, which is positive for autoantibodies, showed a second, a genetic defect in form of a large 84 kb chromosomal deletion. We have identified the chromosomal breakpoints in the DNA of these patients in the Factor H gene cluster on human chromosome 1. This cluster includes several long duplicated regions which are composed of retrotransposal elements and which may cause DNA rearrangements by nonallelic recombination. Defective recombination seems responsible for the genomic deletion and the loss of the *CFHR1* and *CFHR3* genes (Fig. 6). How exactly CFHR1 and CFHR3 deficiency leads to a reduced tolerance and autoantibody formation to Factor H is currently unclear and subject of further investigations. Based on these common features this novel subgroup of aHUS patients is termed DEAP HUS, (**De**ficient for CFHR1/CFHR3 and **a**utoantibody **p**ositive). Apparently DEAP HUS patients respond to a special therapy in form of long term treatment with plasma and immunosuppressive agents.

Absence of Factor H in plasma or expression of a mutant Factor H protein with severe defective complement regulatory functions has been reported in patients with **Membrano-**

**Figure 6**
**Chromosomal deletion of a 84 kB Fragment in the Factor H gene cluster relates to diseases.**
Genetic deletion of the two genes in the Factor H gene cluster CFHR1 and CFHR3 are associated with the risk in the kidney disease HUS, but at the same time show a protective effect for AMD.

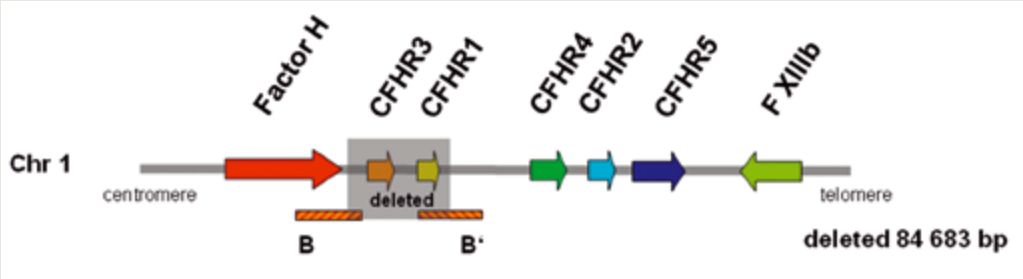



**Figure 7**
**Sequence variations in the Factor H gene in age related macular degeneration (AMD).**
The structure of SCR domain 7 of Factor H is shown. The sequence variation at position 402 which results in an exchange of a Tyrosine residue (green) to a Histidine residue (red) increases the risk for AMD strongly.

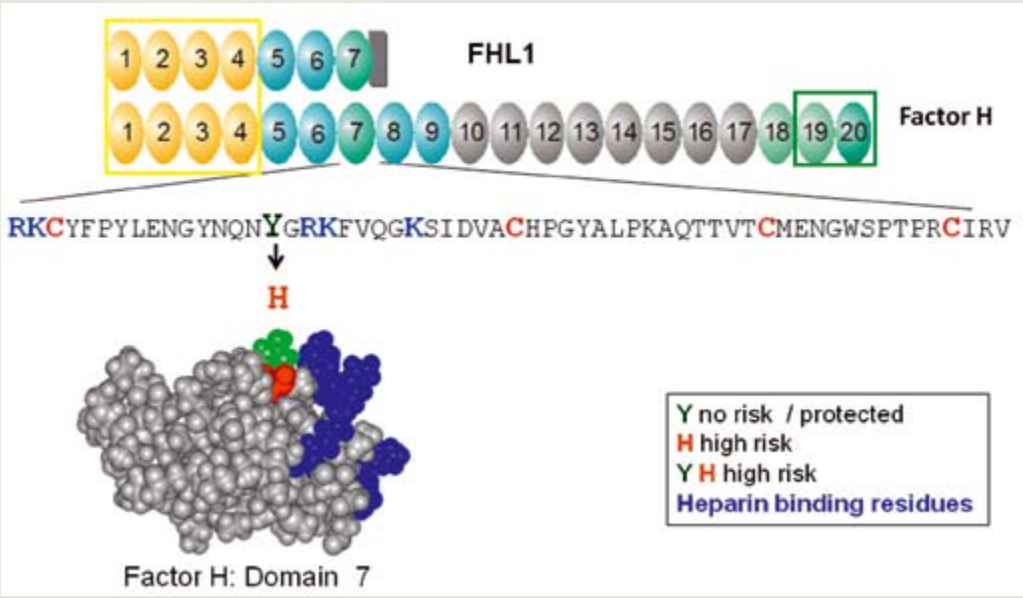
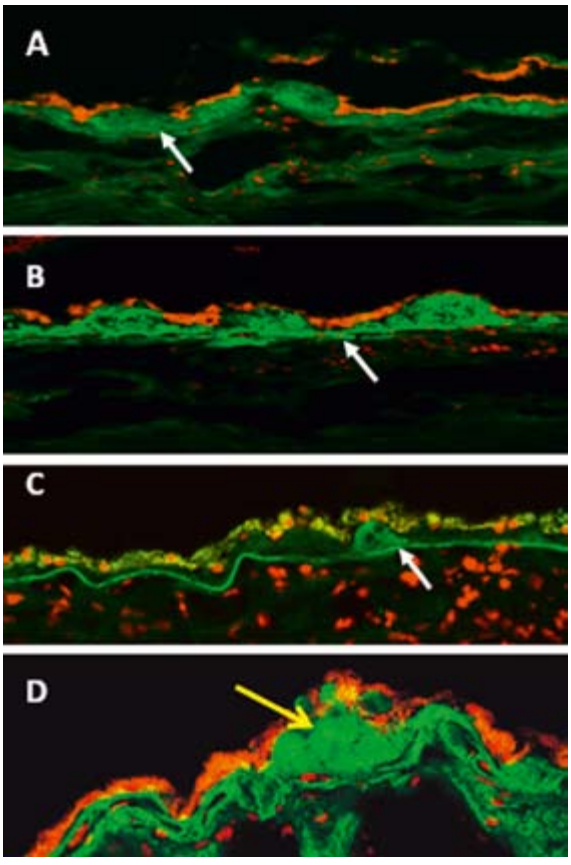



**Figure 8**
**Immunfluorescence staining of Retinal Pigment Epithelial Cells and the Bruch's Membrane of the Eye.**
Patients with AMD develop immune deposits in form of drusen which by immunohistology staining are shown to contain the immune regulator Factor H.




Factor H

FHL 1

CRP

C3c

**proliferative gomerulonephritis type II** (MPGN II). MPGN, also termed dense deposit disease is a rare kidney disease which is defined by a thickening of the glomerular basement, due to deposits within the lamina densa of the glomerular basement membrane. Defective Factor H secretion is caused by mutations which affect central, architectural relevant Cys residues. These changes result in defective protein folding and intracellular processing and cause a block of protein secretion. In addition to several human cases, this type of mutation has been confirmed in animal models, such as Factor H deficient pigs, which represent natural mutants and the first animal model for MPGN, and in Factor H knock out mice, which were genetically designed.

Recently we have identified a second pathomechanism for MPGN II, which is caused by a mutant Factor H protein that is expressed in plasma. Genetic analyses revealed deletion of a single Lys residue (K224), which is located within the complement regulatory region in domain 4 of Factor H. This deletion results in defective complement control: Mutant protein purified from plasma of patients showed severely reduced cofactor and decay accelerating activity, as well as reduced binding to the central complement component C3b. However, cell binding activity of the mutant protein was normal and comparable to wild type Factor H. As both patients, who are daughters of consanguineous parents, and also their healthy mother were positive for C3NeF, the mutant Factor H protein is considered relevant for defective complement control. For both patients replacement with wild type Factor H by fresh frozen plasma was well tolerated, prevented so far disease progression and is over a longer period expected to preserve kidney function.

Currently we have set up a registry for MPGN and have recruited a large number of MPGN patients in Europe. About 65 % of the individuals show defective complement activation or control and genetic analysis are aimed to determine the frequency of Factor H gene mutations of this cohorts and/or to identify additional genes which are associated with this form of kidney disease.

A common polymorphismus such as an exchange of a Tyrosine to Histidine residue at position 402 of Factor H increases the risk for developing **age related macular degeneration** (AMD), which is a common form of blindness in the elderly population of the Western World. We have shown that both Factor H and the Factor H like protein CFHL1, which is encoded by an alternatively spliced transcript derived from the Factor H gene bind to the retinal pigment epithelium/choroid complex in the eye (Fig. 7). In comparison to the protective variant of Factor H (Factor H Y402) the risk variant Factor H H402 binds with lower affinity to the surface of retinal pigment epithelial cells (Fig. 8). This reduced surface binding translates into inappropriate local complement control and defective regulatory activity on the surface of retinal pigment epithelial cells. As a consequence, continuous complement activation is not properly controlled and may lead to a chronic inflammatory state. Later this may lead to formation of immune deposits, epithelial cell separation from the Bruchs membrane and thus results in AMD.

In summary mutations or chromosomal deletions within the Factor H gene cluster affect local control of complement at biological surfaces and result in autoimmune diseases. Understanding the molecular processes in complement regulation helps to define the underlying pathomechanisms of the diverse diseases including HUS, MPGN and AMD. This allows to generate further appropriate diagnostic approaches and new concepts for therapy.

## 4 Technical Support Unit

Group Leader: Susanne Lorenz

### Technical Support Unit for Media Production and Waste Managements

The Technical Support Unit provides media and basic research material for all departments and research groups of the institute. In addition, it utilized sterilization and waste management and inactivation for various biological compounds.

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Deutsche Forschungsgemeinschaft
Schwerpunktprogramm 1160: Kolonisation und Infektion durch humanpathogene Pilze
Teilprojekt: Immune evasion mechanisms of the human pathogenic yeast *Candida albicans*
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Deutsche Forschungsgemeinschaft
Molekulare Genetik und Pathogenese des Hämolytisch-Urämischen Syndroms im Erwachsenenalter
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European Union
6. Forschungsrahmenprogramm der EU
Integrated Project: New medicines for tuberculosis (NM4TB)
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National Institutes of Health, Bethesda, USA
Collaborative Study of Membranoproliferative Glomerulonephritis Type II
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Targeted Complement Inhibition as a Therapeutic Approach for Dense Deposit Disease
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Pro Retina - Stiftung zur Verhütung von Blindheit, Germany
Die Rolle des Komplementsystems, insbesondere der beiden Komplementregulatoren Faktor H und FHL-1, bei der AMD des Auges
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## Selected publications
(HKI authors in bold)


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# Department of Microbial Pathogenicity Mechanisms

# Department of Microbial Pathogenicity Mechanisms

The **Department of Microbial Pathogenicity Mechanisms (MPM)** was established in the spring of 2007 and is concerned with the investigation of infections caused by human pathogenic fungi. Research is focused on the pathogenesis of mycoses due to the yeasts *Candida albicans* and *C. glabrata*.

### *Candida* Infections

In contrast to most pathogenic fungi of humans, such as *Aspergillus fumigatus*, *Cryptococcus neoformans* and *Histoplasma capsulatum*, which are found in the environment, *Candida albicans* and *C. glabrata* belong to the normal microbial flora of the skin, intestinal tract or other mucosal surfaces, and are regarded as harmless commensals under most circumstances. In fact, these commensal yeasts can be detected in up to 71 % of the healthy population (depending on the methods of sample collection and the body sites) (Ruhnke, 2001).

An intact immune system and a balanced microbial flora are normally sufficient to protect an individual from *Candida* infections. However, certain critical events such as extensive antibacterial treatment or immune system dysfunction may enable these fungi to overgrow the microbial flora on mucosal surfaces (superficial candidosis). In hospital settings, *Candida* species may cause life-threatening systemic and invasive infections in a growing population of vulnerable patients. In fact, bloodstream infections due to *Candida* (candidaemia) are associ-

## INTRODUCTION | EINLEITUNG

Head:
Prof. Dr. Bernhard Hube

Die **Abteilung Mikrobielle Pathogenitätsmechanismen (MPM)** wurde im Frühjahr 2007 gegründet und erforscht die Ursache und Entwicklung von Infektionen durch humanpathogene Pilze. Die Untersuchungen konzentrieren sich dabei auf die Pathogenese von Pilzinfektionen durch Hefen, insbesondere *Candida albicans* und *C. glabrata*.

### *Candida*-Infektionen

Im Gegensatz zu vielen anderen krankheitserregenden Pilzen des Menschen, wie zum Beispiel *Aspergillus fumigatus*, *Cryptococcus neoformans* und *Histoplasma capsulatum*, welche normalerweise nur in der Umwelt zu finden sind, zählen *Candida albicans* und *C. glabrata* zur normalen mikrobiellen Flora der Haut, des Verdauungstraktes oder anderer Oberflächen mit Schleimhäuten und gelten in der Regel als harmlose Kommensale. Tatsächlich können diese kommensalen Hefen in bis zu 71 % der gesunden Bevölkerung gefunden werden (abhängig von den Untersuchungsmethoden und der Körperregion) (Ruhnke, 2001).

Ein intaktes Immunsystem und eine gesunde mikrobielle Flora sind normalerweise ausreichend um einen Menschen vor einer *Candida*-Infektion zu schützen. Unter bestimmten Umständen, zum Beispiel nach antibakteriellen Behandlungen oder bei Fehlfunktionen des Immunsystems, kann der Pilz die normale mikrobielle Flora auf Schleimhautoberflächen überwuchern (oberflächliche Candidosen). In einer stetig anwachsenden Anzahl von gefährdeten Patienten, die in Krankenhäusern behandelt werden, können *Candida*-Arten sogar lebensbedrohliche systemische und invasive Infektionen verursachen. Tatsächlich ist die Letalitätsrate bei Blutinfektionen durch *Candida* (Candidämie) höher als bei

ated with the highest crude mortality rate of all bloodstream infections, even higher than those caused by bacteria (Perlroth et al., 2007). *Candida* cells may enter the bloodstream by direct penetration from epithelial tissues, due to damage of barriers in the body caused by surgery, polytrauma or drug treatment, or may spread from biofilms produced on medical devices. From the bloodstream, cells can infect every organ of the body (invasive candidosis), but appear to prefer certain organs depending upon the route of infection (Kullberg et al., 2002). Only relatively minor immunosuppression is required to predispose to invasive *Candida* infections, and *Candida* is by far the most common cause of nosocomial (hospital acquired) fungal infections (Perlroth et al., 2007).

The biomedical importance of *Candida* and other fungal infections has recently been recognised by several research groups in Europe. Their projects are supported by different transnational programmes of the European Commission and national science foundations. Some nine European networks focussing on molecular medical mycology and the training of young researchers have been established during the 6th framework programme and the ERA-NET PathoGenoMics scheme (Hube, 2007).

### *Candida albicans*

The yeast *C. albicans* is regarded as the most important of all medically relevant fungi and is an extremely successful pathogen of human beings. *C. albicans* is a polymorphic yeast

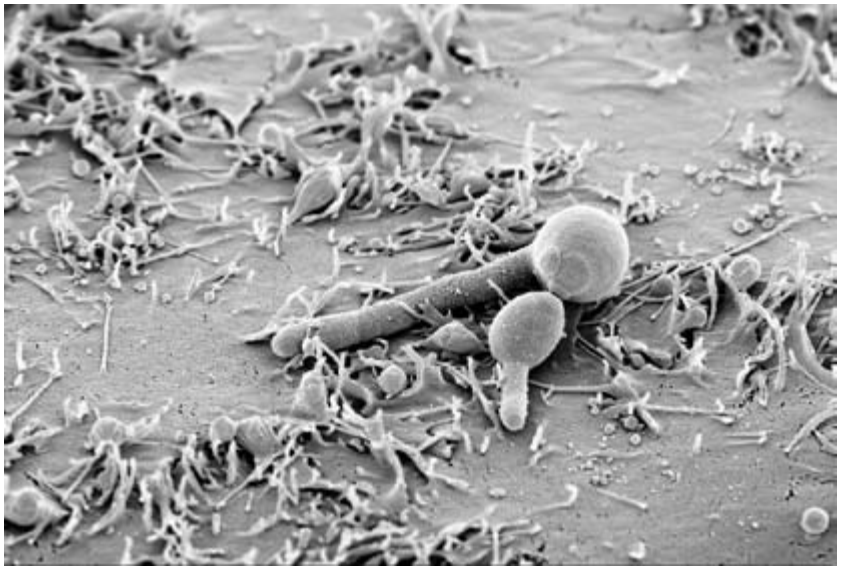
bakteriellen Infektionen (Perlroth et al., 2007). *Candida*-Zellen können das Blutgefäßsystem durch direkte Penetration von den Epithelschichten, nach Zerstörung physikalischer Barrieren durch chirugische Eingriffe, Polytrauma oder medikamentöse Behandlungen, oder durch Verbreitung ausgehend von Biofilmen auf implantierten medizinischen Hilfsmitteln (z. B. Katheter), erreichen. Von den Blutgefäßen aus können die Pilzzellen praktisch jedes Organ des Menschen infizieren (invasive Candidosen), sie scheinen jedoch, je nach Infektionsroute, bestimmte Organe zu bevorzugen (Kullberg et al., 2002). Bereits relativ geringe Immunosuppressionen reichen aus, um einen Menschen für eine invasive Candidose empfänglich zu machen und *Candida* gilt als der bei weitem häufigste Erreger von nosokomialen (im Krankenhaus erworbenen) Pilzinfektionen (Perlroth et al., 2007).

Die biomedizinische Bedeutung von *Candida* und anderen Pilzinfektionen wurde mittlerweile von mehreren Forschergruppen in Europa erkannt. Die Projekte dieser Gruppen werden durch unterschiedliche nationale und internationale Programme verschiedener nationaler Wissenschaftsinstitutionen und der Europäischen Union unterstützt. Immerhin neun Europäische Netzwerke, die sich auf molekulare medizinische Mykologie und der Ausbildung junger Nachwuchswissenschaftler konzentrieren, wurden durch das sechste Rahmenprogramm der EU und das ERA-NET PathoGenoMics Programm gefördert (Hube, 2007).

### *Candida albicans*

*Candida albicans* gilt als der medizinisch wichtigste Pilz und ist ein extrem erfolgreicher Pathogen des Menschen. *C. albicans* ist eine polymorphe Hefe, die zur normalen

**Figure 1**
Germinating *Candida albicans* cells on human epithelial tissue (from D'Enfert C, Hube B (2007) *Candida*: Comparative and Functional Genomics. Horizon Bioscience; Caister Academic Press)



which belongs to the normal microbial flora of human beings. Oral and vaginal infections with *C. albicans* are extremely common in even mildly immunocompromised individuals. Furthermore, in severe cases, *C. albicans* penetrates into deeper tissue and may enter the bloodstream. From the bloodstream, the fungus has the potential to invade almost all body sites and organs, causing life-threatening systemic infections that requires the adaptation to a variety of different environmental stresses. Therefore, *C. albicans* is able to survive and proliferate in radically changing environments with drastic changes in oxygen and carbon dioxide, pH, osmolarity, availability of nutrients and temperature. In addition it has to resist the constant surveillance of the immune

mikrobiellen Flora des Menschen gehört. Selbst bei Menschen mit einem nur gering geschwächten Immunsystem kommen häufig orale und vaginale *C. albicans* Infektionen vor. Bei stärkereren Störungen kann es dazu kommen, dass *C. albicans* in tiefere Gewebeschichten vordringt und von dort sogar das Blutsystem erreicht. Aus dem Blut kann *C. albicans* in die meisten Organe eindringen und so lebensbedrohliche systemische Infektionen hervorrufen. Während dieses Prozesses muss sich *C. albicans* an unterschiedlichste Umweltbedingungen anpassen – dazu gehören Schwankungen im Sauerstoff- und Kohlendioxidgehalt, dem pH-Wert, der Osmolarität, den verfügbaren Nährstoffen und der Temperatur. Außerdem muss der Pilz den Angriffen des Immunsystems widerstehen. Nur wenige Mikroorganismen sind wie *C. albicans* in der Lage, an so vielen verschiedenen Stellen des Körpers Infektionen hervorzurufen. Durch die vor kurzem vollendete Sequenzierung des *C. albicans*-Genoms, die Verfügbarkeit von genomweiten Mikroarray-Chips und die Entwicklung von Techniken, die relativ einfach schnelle molekularbiologische Manipulationen des *C. albicans*-Genoms ermöglichen, wurden in den letzten Jahren eine Fülle von Informationen über die fazinierende Biologie und die Virulenzmechanismen dieses Pathogen generiert (Berman et al., 2002). Darüber hinaus wurde eine Reihe von Infektionsmodellen etabliert, um genomweite Genexpressionen oder die Beteiligung von ausgesuchten Genen bei Infektionen untersuchen zu können (Schaller et al., 2006; Thewes et al., 2007; Zakikhany et al., 2007).

### *Candida glabrata*

*C. glabrata* gilt als „emerging pathogen", der immer häufiger in Kliniken isoliert wird. In

system. Few pathogenic microorganisms have the potential to cause infections at such a broad range of body sites.

The recent completion of the *C. albicans* genome sequence, the availability of whole-genome microarrays and the development of tools for rapid molecular-genetic manipulations of the *C. albicans* genome are generating an explosion of information about the intriguing biology of this pathogen and about its virulence mechanisms (Berman et al., 2002). In addition, a number of infection models have been established to investigate aspects of *Candida* infections including genome wide genes expression and the contribution of selected gene during experimental infection (Schaller et al., 2006; Thewes et al., 2007; Zakikhany et al., 2007).

***Candida glabrata***

*C. glabrata* has been recognised as an emerging pathogen, with increasing numbers of clinical isolates. In many cases *C. glabrata* is the second most prevalent pathogenic yeast in humans after *C. albicans*. Importantly, this fungus has a naturally high resistance to most commonly used antifungal agents. Yet, relatively few studies have dealt with the pathogenicity mechanisms of *C. glabrata*.

Three attributes make *C. glabrata* an attractive model organism for fungal infections: *C. glabrata* is non-filamentous, has a haploid genome and is very closely related to the non-pathogenic bakers yeast *Saccharomyces cerevisiae*. In fact, the sequencing of the *C. glabrata* ge-

vielen Fällen ist *C. glabrata* nach *C. albicans* mittlerweile die zweithäufigste pathogene Hefe, die beim Menschen gefunden wird. Ein wichtiger Faktor dabei dürfte die natürliche hohe Resistenz gegen die gebräuchlichsten Antimykotika sein. Trotzdem gibt es relativ wenig Studien, die sich mit den Pathogenitätsmechanismen von *C. glabrata* befassen. Drei Eigenschaften machen *C. glabrata* zu einem attraktiven Modellorganismus für Pilzinfektionen: *C. glabrata* ist ein nichtfilamentöser Pilz, hat ein haploides Genom und ist ein naher Verwandter der klassischen Bäckerhefe *Saccharomyces cerevisisae*. Die Sequenzierung des *C. glabrata*-Genoms brachte verblüffende Ähnlichkeiten mit *S. cerevisiae* zutage (Dujon et al., 2004). Viele genomische und molekularbiologische Werkzeuge, wie z. B. Promotoren, Markergene, Reportergene und selbst Plasmide können in beiden Hefen verwendet werden.

**Untersuchungen zu Pathogenitätsmechanismen von *Candida***

Ziel der Forschungen der Abteilung MPM ist es, am Beispiel von *Candida*-Hefen aufzuklären, welche Faktoren krankheitserregende Pilze benötigen, um Infektionen zu verursachen und welche Mechanismen bei den Interaktionen von *C. albicans* und *C. glabrata* mit dem Wirt Krankheiten auslösen. Dabei kommen zelluläre, mikrobiologische, molekulare und biochemische Methoden zum Einsatz. Durch die Erforschung der Ursachen der Pathogenität sollen nicht nur die Pathogenitätsmechanismen besser verstanden, sondern auch Ansatzpunkte für neue Medikamente gefunden werden.

**Infektionsmodelle zur Untersuchung pathogener Pilze**

Eingebettet in die Abteilung MPM ist die **Forschungsgruppe „Infektionsmodelle**

nome uncovered many striking similarities to *S. cerevisiae* (Dujon et al., 2004). Many genomic and molecular biology tools such as promoters, markers, reporters and even plasmids are interchangeable between the two yeasts. The known genome sequence of *C. glabrata* and microarray techniques provide a solid basis for advanced molecular analysis of pathogenicity traits and mechanisms.

**Analysis of the Pathogenicity Mechanisms of *Candida***

Using cellular, microbial, molecular and biochemical methods and *C. albicans* or *C. glabrata* as model organisms, the goal of the department of MPM is to identify factors which fungal pathogens need in order to cause disease. In addition to these efforts to increase our understanding of the basics of pathogenesis of fungal infections, the department also seeks to identify new potential targets for antimycotic drug development.

**Infection Models for Fungal Infections**

Integrated into the department of MPM is a research group which carries out experimental fungal infections using *in vitro, ex vivo* and in *vivo* infection models.

Dr. Albert Härtl led the group until his retirement in May 2007 and was succeeded by Ilse Jacobsen, PhD, a veterinarian specialized in Microbiology, in April 2007.

**und Wirkstoffprüfung“**. Forschungsschwerpunkte dieser Gruppe liegen auf der experimentellen Infektionsbiologie und auf toxikologischen und pharmakologischen Untersuchungen von Naturprodukten.

Bis zu seinem Ruhestand im April 2007 wurde die Gruppe durch Dr. Albert Härtl geleitet. Als seine Nachfolgerin ist Ilse Jacobsen, PhD, Fachtierärztin für Mikrobiologie, seit April 2007 am HKI tätig.

Zur Untersuchung der Virulenz von *A. fumigatus* und *C. albicans* setzt die Gruppe etablierte Mausmodelle ein. Murine Infektionsmodelle für *A. terreus* und *C. glabrata*, zwei weitere humanpathogene Pilze, an denen am HKI geforscht wird, werden zur Zeit etabliert. Traditionell wird die Virulenz von Pilzmutanten anhand der Mortalität infizierter Versuchstiere beurteilt. Um jedoch zu verstehen, warum bestimmte Mutationen die Virulenz vermindern, sind tiefergehende Untersuchungen notwendig. Mittels Histopathologie können auf zellulärer Ebene Einblicke in den Infektionsprozess gewonnen werden. Daher wurde eine Kooperation mit dem am Beutenbergcampus ansässigen Fritz-Lipmann-Institut (FLI) etabliert. Mit Hilfe der technischen Ausstattung am FLI werden aus Gewebeproben histologische Präparate angefertigt, die dann von der Gruppe ausgewertet werden. Als zusätzliche Informationsquelle über die Prozesse *in vivo* werden Parameter der klinischen Chemie und Blutenzymwerte herangezogen.

Als Alternative zu Tierversuchen wird das Modell des embryonierten Hühnereis verwendet. Dieses Modell wird zur Zeit gezielt weiterentwickelt, um es zur Untersuchung der Pathogenese von Pilzinfektionen einsetzen zu können.

Infection models in mice are routinely used to study virulence of *A. fumigatus*, *C. albicans* and *C. glabrata* mutant strains. Murine models for *A. terreus*, an additional fungal pathogen studied by groups of the HKI, are currently being established. Survival is the traditional readout parameter for most *in vivo* models; however, in depth post mortem analysis is necessary to dissect the underlying mechanisms leading to attenuation of defined mutants. Histopathology is a useful way to gain information on the cellular processes during infection. Therefore, a collaboration with the Fritz Lipmann Institute (FLI) at the Beutenberg Campus was established to process tissue samples for histopathology, which are then analysed by the group. Additionally, organ damage is monitored by measuring blood levels of marker enzymes.

Embryonated hen's eggs are another valuable alternative model for studying host-fungal interactions. This model is being further developed to study the infection process of fungal pathogens.

As an essential part of the "Internal Product Line" the group also performs toxicological, pharmacological and immuno-pharmacological assays with novel natural products and their synthetic derivatives. The group thereby contributes to the biological profiling of these substances.

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Innerhalb des HKI trägt die Gruppe zudem zur Durchgehenden Bearbeitungsrichtlinie (DBL) bei. In diesem Rahmen werden toxikologische, pharmakologische und immunologisch-pharmakologische Untersuchungen durchgeführt, die der biologischen Charakterisierung neuartiger Naturstoffe und ihrer Derivate dienen.

## 1 *Candida albicans* Interactions with Human Epithelial Cells

Group Leaders: Ronny Martin,
Betty Wächtler, Bernhard Hube

*Candida albicans* is the most common oral fungal pathogen of humans, but the mechanisms by which this fungus invades and persists within mucosal epithelium are not clear. Normally, a harmless commensal of mucosal surfaces in healthy individuals, *C. albicans* frequently causes oral infections in HIV infected and otherwise immunocompromised patients. During the most common type of oral infections, oropharyngeal candidosis, the fungus invades the oral mucosa and persists within the epithelium causing superfical lesions. Usually, fungal proliferation, colonization and invasion are hindered by dense epithelial layers with high turnover rates and innate defence mechanisms such as secretion of antimicrobial compounds. However, *C. albicans* has developed strategies to overcome these defence mechanisms for both commensal growth and infection. One of the strategies include the morphological switch from yeast cells to filamentous hyphae and the virulence attributes associated with the transition.

In this DFG-funded project we study the interactions between *C. albicans* and host cells during the different stages of oral infections to identify factors, which are associated with infection and to elucidate the function, relevance and role of these factors.

In collaboration with Julian Naglik (King's College London) and Martin Schaller (University of Tübingen), our group studied the different stages of an experimental oral infection with *C. albicans*, using a reconstituted human oral epithelium (RHE, Schaller et al., 2007). Therefore we combined histological, microscopcial, transcriptional and molecular approaches to examine the interactions of *C. albicans* with oral epithelial cells. Oral infections were dissected into three phases: an early attachment phase (1-3 h), an invasion phase (3-12 h) and a late phase (more than 12 h) which is characterized by strong tissue damage. In the early phase, the initial physical contact between the epithelium and fungal cells stimulated a switch to the hyphal form (> 98% within 1 h) and subsequent adherence to the epithelium. The following invasion phase was characterized by invading hyphae and correlated with moderate tissue damage in the mid phase (6 h) and severe tissue destruction in the later phase (more than 12 h). Based on histological, cellular and kinetic observations we predicted that the expression profiles of the early phase (1–3 h) reflected the initiation of infection, and the late phases (12–24 h), the maintenance and progression of the disease. For each infection phase we examined the transcriptional profile of *C. albicans* with DNA microarrays and observed stage-specific gene expression. While in the early phase, genes required for hyphal formation are dominantly up-regulated, genes involved in the response to several stresses (change of carbon sources, limited nitrogen sources, nitrosative stress) were up-regulated in the late phases.

To determine whether our experimental RHE model observation were consistent with *in vivo* infections, we compared the transcriptional profiles of *C. albicans* from the RHE with those from *C. albicans* derived from samples of HIV patients suffering from oral candidosis. By doing so, we identified an unknown function gene which was up-regulated in both RHE and patients. A mutant without this gene was neither able to form hyphae under all tested *in vitro* conditions or cause destruction of host tissues. However, the mutant displayed a very interesting phenotype during its interactions with epithelial host cells. In contrast to the *in vitro* observations, the mutant was able to form short hyphae after contact to epithelial cells. These hyphae allowed entry of the mutant into host cells. However, after invasion the mutant

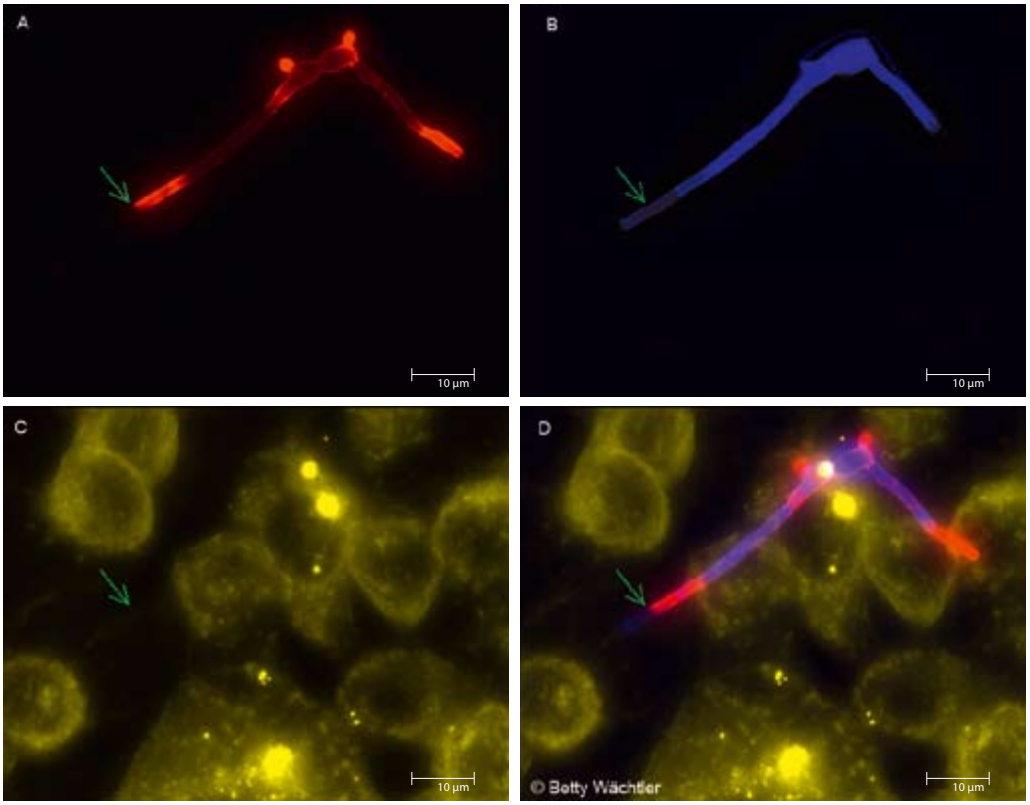


**Figure 2**
*Candida albicans* interactions with human epithelial cells. By using different fluorescent dyes and antibodies we are able to show how *C. albicans* cells invade human host cells. An anti-*Candida* antibody does only stain hyphal parts which are outside of the host cell (A), after permeabilization the fluorescent dye Calcofluor White stains those fungal parts which are inside or outside of human cells (B). The host cell membrane is stained with a lipophilic tracer (C). The overlay of the single pictures shows that *C. albicans* hyphae not only invade single host cells but also penetrate straight through the host cells and disseminate and invade the next host cells (D). The arrow indicates the penetration into the second host cell.

could not maintain filamentous growth and switched back to yeast cells which persisted in the upper layers of the host tissue and did not disseminate into lower tissue sections like the hyphae of a wild type strain. Therefore, the gene was named EED1 (**E**pithelial **E**scape and **D**issemination). In conclusion, we could identify an infection-associated gene with previously unknown function which is required for the maintenance of filamentous growth (Results of this project part were reported in Zakikhany et al., 2007).

Invasion of oral epithelial cells by *C. albicans* is a characteristic step in oropharyngeal candidosis and is necessary to damage these cell types *in vitro*. To understand how *C. albicans* is able to invade oral epithelial cells, another part of the project deals with the mechanisms by which *C. albicans* enters these cells.
One process already described is called "induced endocytosis". Binding of cell surface components of *C. albicans*, such as the protein Als3, which was recently identified as a fungal invasin, to host cell surface proteins such as cadherins led to actin rearrangements and subsequent engulfment of the fungus. A second mechanism is active penetration, supported by extracellular hydrolytic activity of the fungus. *In vitro*, it is possible to differentiate between these two processes, and in our studies we have begun to uncover which fungal factors are involved in the two processes. In collaboration with Frederic Dalle (University of Dijon), we examined the interactions of *C. albicans* with epithelial cells (cell line TR-146) and enterocytes (cell line Caco-2). The fungus is able to adhere to, invade into and damage these cells, however the fungus is more adherent and invasive during interactions with the oral epithelial cell line than with enterocytes. In our studies we observed induced endocytosis with oral epithelial cells, but not with enterocytes, indicating that there are differences in the interactions of *C. albicans* with different host tissues.

**References**
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*Cell Microbiol* 9, 2938–54.

It is possible to discriminate between the two processes of *C. albicans* internalisation at the cellular level. Induced endocytosis requires viable host cells and a functional actin cytoskeleton – even killed fungal hyphae were internalised by viable host cells. Especially in the early phases of infection (1h) this process plays a critical role. At later time points, active penetration seems to be the major process of invasion and is independent from the viability of the host cells.
In a screening of several *C. albicans* mutants, we observed that strains lacking key regulators of hyphal growth such as Efg1, Ras1 or Hgc1 were not invasive at all – neither by induced endocytosis or active penetration. Als3, a known *C. albicans* invasin, was observed to be almost essential for induced endocytosis at early time points of infection but in later stages, importance of this protein decreased. We also identified mutants which, although able to form hyphae, had reduced invasive potential and caused reduced host cell damage. These mutants are currently under examination to identify fungal factors which contribute to invasion of epithelial cells by *C. albicans*.

Financed in part by the "Deutsche Forschungsgemeinschaft". Project "Identification and characterisation of virulence associated genes during oral infections with *Candida albicans*" within the DFG-priority program SPP1160 Förderkennzeichen: HU 528/10-1 and 10-2.

## 2 Infection-associated Genes of *Candida albicans*

Group Leaders: François Mayer, Duncan Wilson, Bernhard Hube

### Infection-associated Genes

Transcriptional profiling is a powerful tool for dissecting the molecular mechanisms that an organism employs in response to specific environments. We have taken advantage of this technology to unravel the biology underlying *C. albicans* infections. By analysing the transcriptome of this pathogen during various types of infection, we have begun to dissect which genes, regulons and biological processes are relevant during the infection process. Gene expression data has provided evidence about the types of microenvironments that the fungus encounters during infection of the host. For example, during liver infection, key enzymes of glycolysis, acetyl-CoA biosynthesis and the TCA cycle were up-regulated, suggesting that *C. albicans* is able to utilise sugars during infection of this organ. On the other hand, within oral tissue, it appears that glucose is not available, as indicated by the up-regulation of components of gluconeogenesis and the glyoxylate cycle. Although capable of providing such detailed evidence about the pathogen's behaviour *in vivo*, a major problem still exists in the interpretation of such datasets: usually, a large proportion of significantly regulated genes are of unknown function. This problem is compounded by the fact that most annotation of the *C. albicans* genome has been achieved based on homology with the non-pathogenic yeast, *Saccharomyces cerevisiae*. We therefore hypothesised that genes of unknown function, which are specifically expressed during infection ("infection-associated (IA) genes") are likely to be involved in host-pathogen interactions. Therefore, in this project, IA-genes were scrutinised *in silico* using bioinformatics tools to search for functional domains, localisation signals and relatedness to other species. Based on these analyses, a set of 50 genes with a high likelihood of involvement in host-pathogen interactions were selected for further investigation. In order to gather detailed information about the roles of these genes during host-pathogen interactions, a number of approaches were taken. Primarily, expression of IA-genes under different conditions was analysed in detail using GeneSpring software. Using this technique, we were able to define the transcriptional modularity of *C. albicans* during clinically relevant events and characterize gene sets that are co-expressed under specific subsets of conditions. By combining information derived from these regulons with additional functional genomic data, we were able to begin to assign novel functional annotations for these uncharacterised genes. For further characterisation, mutants were generated with specific inactivations of

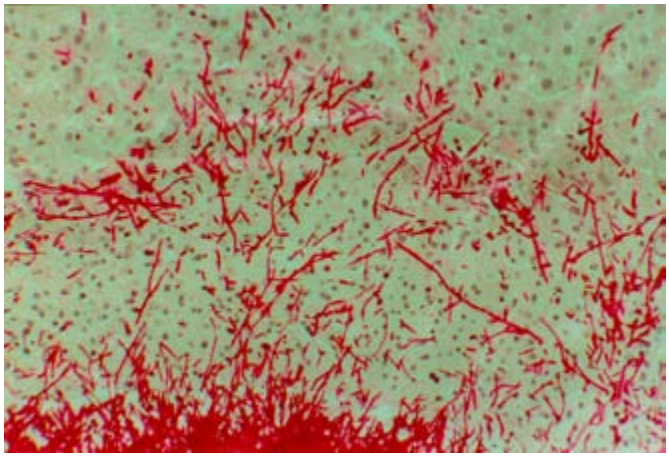

**Figure 3**
Invasion of *Candida albicans* into liver tissue (Kretschmar et al. (1999) *Infect Immun* 67, 6637–6642).

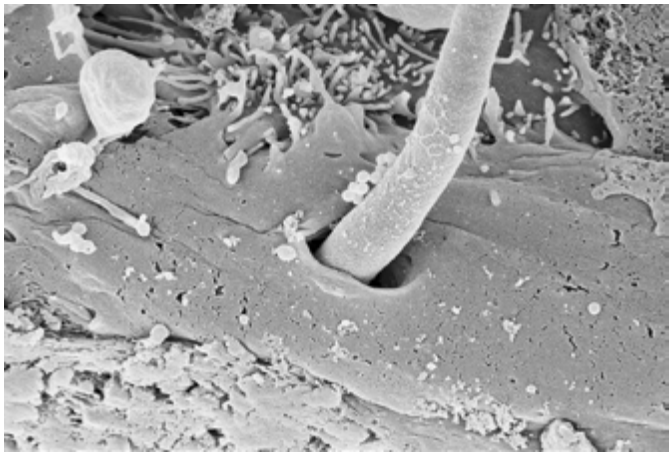

**Figure 4**
Active penetration of *Candida albicans* into a human host cell (Zakikhany et al. (2007) *Cell Microbiol* 9, 2938–2954).

infection-associated genes. Because *C. albicans* is diploid and lacks a fully defined sexual cycle, classical genetics cannot be performed with this organism and advanced molecular biology approaches are required for genetic characterisation. The disruption strategy we employ is based on a rapid PCR method (Gola et al., 2003) using strain BWP17, which is auxotrophic for arginine, histidine and uridine. First, ARG4- and HIS1-disruption cassettes were generated using primers containing approximately 100 bp homology to the immediate upstream and downstream sequences of the relevant target gene. The ARG-disruption cassettes were then used in a primary transformation event to disrupt the first copy of the relevant gene of interest by homologous recombination. Heterozygotes were then transformed in a second transformation process with the HIS-disruption cassettes. Correct deletion of both alleles of the target gene was verified by diagnostic PCR and Southern blot analysis. In a final step, the remaining uridine auxotrophy of the homozygous mutant can be used to restore a wild type copy of the gene of interest, thus satisfying Molecular Koch's postulates.

The pathogenicity of *C. albicans* relies on a range of virulence factors and fitness attributes such as the yeast-to-hyphal transition, invasiveness, expression of adhesins, secretion of hydrolytic enzymes, cell wall biogenesis, essential nutrient acquisition, together with metabolic flexibility and adaptation to stress. For these reasons, our set of IA-gene mutants were first tested *in vitro* for their ability to form hyphae, invade agar, utilise alternative nutrient sources and respond appropriately to various stresses. Next, mutants were assayed in defined infection models for their ability to adhere to and damage various types of human cells. By analysing the behaviour of our mutants in both classical *in vitro* conditions and in defined infection models we are able to both assign biological function

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whilst simultaneously determining the role of the gene product during infection. Presented in the following paragraphs, are examples of three unknown function genes, the biological function and role during infection of which have begun to be determined.

### *OCS5* is Part of a Virulence-associated Gene Family and is Required for Utilisation of Alternative Carbon Sources at Physiological Temperature

Highly expressed during experimental oral candidosis, *OCS2* was shown to encode a putative secreted protein with four other close homologues in the *C. albicans* genome. Using advanced bioinformatics tools, we phylogenetically mapped the relationship of the five family members, assigned novel regulon membership and successfully predicted transcription factor binding site locations in the respective promoter regions. Furthermore, the mutant lacking *OCS2* was unable to grow with acetate or ethanol as the sole carbon source at physiological temperature. This finding indicates a role for *OCS2* during growth within the host, as other mutants lacking components of the glyoxylate cycle exhibit reduced survival in macrophages and are avirulent in a mouse model of systemic candidosis (Ramírez et al., 2007).

### *OPK1* Encodes a Putative Kinase Required for Adhesion to Oral Epithelial Cells

Transiently expressed during initial contact with the oral epithelium, it was predicted that *OPK2* would encode a protein involved in early interactions with oral tissue. In agreement with this prediction, we demonstrated that deletion of *OPK2* resulted in impaired adhesion to an oral epithelial monolayer but did not attenuate tissue damage in this model. However, analysis of the predicted protein sequence did not suggest that *OPK2* encoded an cell adhesin, but rather a protein kinase, suggesting a role for *OPK2* in the regulation of adhesion factors during oral infection.

### *OCS2* Encodes a Predicted Membrane Protein Required for Invasive Hyphal Development and Oral Epithelial Tissue Damage

*OCS2* was identified due to its high expression late in experimental oral infection and *in vivo* samples from HIV patients suffering from oral candidosis, suggesting a role for this gene in the persistence of oral infections. *In silico* analysis predicted the presence of eight transmembrane helices. Its deletion resulted in no apparent growth defects and normal hyphal development was observed under a number of induction conditions; however, the mutant was unable to produce invasive hyphae upon embedding in an agar matrix and was significantly attenuated in its ability to damage an oral epithelial monolayer. Taken together, these results suggest that *OCS2* encodes an integral membrane protein required for invasive growth within host tissue.

Financed in part by the FP6 Marie Curie Research Training Network "Interaction of fungal pathogens with host cells: a post-genomic approach; GALAR FUNGAIL 2" Förderkennzeichen MRTN-CT-2003-504148.

## 3 Infection-associated Genes and Molecular Biology of Pigment Production of *Candida glabrata*

Group Leaders: Katja Seider, Sascha Brunke, Bernhard Hube

The principal aim of this project is to study the important human fungal pathogen, *Candida glabrata*, so as to better understand the molecular mechanisms of its pathogenicity.
*C. glabrata* is part of the normal microbial flora of human mucosal surfaces. Like *C. albicans*, it is considered to be a human commensal but can turn into a successful opportunistic pathogen, being the second-most frequent cause of systemic mycoses – accounting for up to 20 % of clinical mycoses. The high risk of *C. glabrata* infections arises from the intrinsically high drug resistance to widely used antifungal drugs, therefore replacing other *Candida* species in patients receiving drug treatment. Indeed, efficacy of treatment of *C. glabrata*

infections has not improved during the last decade.

Besides drug resistance, only few virulence attributes, such as adhesion factors and phenotypic switching have been characterised, and infection mechanisms remain poorly understood. Furthermore, key aspects in *C. albicans* tissue invasion and persistence, most notably filamentation through hyphal formation and secretion of proteases, have no parallels in *C. glabrata*. Nevertheless, despite a lack of filamentation, *C. glabrata* is invasive *in vivo* and can cause severe infections, mainly in immunocompromised patients.

We hope to address the question of how *C. glabrata* colonises and causes disease and which factors contribute to different stages of the infection process. Therefore, we started analysing different steps of fungus-host interactions – from attachment and invasion of epithelial cells to tissue destruction and interactions with immune cells.

### *Candida glabrata* Interactions with Human Epithelial Cells

Usually the first step of infections is to overcome dense epithelial layers which hinder fungal proliferation, colonization and invasion through high turnover rates and the secretion of antimicrobial peptides. We have already described two mechanisms that *C. albicans* uses to invade: induced endocytosis and active penetration. On this basis we examined the interactions between *C. glabrata* and the epithelial cells line, TR-146. Because *C. glabrata* is non-filamentous, we were not able to detect any active penetration, but still observed intracellular (invading) yeast cells. In fact, this invasion could be blocked by incubation with Cytochalasin D, an inhibitor of actin cytoskeleton rearrangements, suggesting that the observed invasion is based on induced endocytosis. This may be how *C. glabrata* adheres and invades during both commensal growth and infection. Since active penetration is responsible for the majority of invasion by *C. albicans*, it was not surprising to find that tissue damage caused by *C. glabrata* is about three times lower than by *C. albicans*.

### *Candida glabrata* Interactions with Human Cells of the Innate Immunity

Although we still do not know how *C. glabrata* disseminates in immunocompromised patients, there exist routes – either via invasion of epithelial tissues or due to damage of barriers in the body caused by surgery, polytrauma or drug treatment – to enter the bloodstream, distribute and infect deeper organs. During penetration of tissue and the dissemination through the bloodstream, *C. glabrata* is always exposed to cells of the innate immunity. Consequently, *C. glabrata* must have developed strategies to counteract or bypass mammalian host defence systems, thereby enabling the fungus to cause systemic disease.

Hence a further part of the project examines interactions with mammalian cells implicated in innate immunity such as macrophages and neutrophils. In contrast to *C. albicans*, relevant host immune defences against *C. glabrata* are unknown. We have begun to look at the general phagocytic pathway of *C. glabrata* in cultured and primary macrophages and observed that *C. glabrata* survives and even replicates in macrophages. It is an enticing possibility that the fungus uses macrophages to hide from other immune cells. The next step will be to localize the replicating yeast cells within compartments of the macrophages. It is possible that macrophages are unable to kill *C. glabrata* and that, as is the case for *C. albicans*, neutrophils play a more important role. Indeed, it has been demonstrated that reactive oxygen species generation by neutrophils in response to *C. glabrata* is higher than in response to *C. albicans*.

In addition to analysis of general interactions of *C. glabrata* with immune cells, we are also searching for virulence genes that are necessary and sufficient to help evading the host immune defence. Using a transposon insertion mutant library, kindly provided by Prof. Brendan Cormack (Johns Hopkins University School of Medicine, Baltimore, USA), we aim to identify mutant strains showing significantly reduced survival in macrophages. Therefore, we established a high through-put screening procedure. Mutants, that do show an altered survival rate, will be further characterized in a number of *in vitro*, *ex vivo* as well as *in*

*vivo* models to elucidate the physiological relevance of the disrupted gene. These models include cultured as well as primary cells, the reconstituted human epithelium (RHE) tissue and mouse models. Because *C. glabrata* and *Saccharomyces cerevisiae* are so closely related, the non-pathogenic bakers yeast can serve as a useful control. Of particular interest for future consideration are the several hundred *C. glabrata* genes without homologues in *S. cerevisiae*.

### Molecular Biology of Pigment Production of *Candida glabrata*

Pigments can be found in many different pathogenic fungi, ranging from *Aspergillus* species to *Cryptococcus neoformans*. In most cases pigmentation is due to melanin, which is able to protect the fungal cells from diverse environmental stresses. With its protective function against oxidants and hydrolytic enzymes, melanin can play an important role in surviving the immune response of the host.

The yeast *C. glabrata* also forms pigment, consisting of a mixture of indole-derived compounds. It is synthesized when certain indolic compounds are provided as precursors, as is the case with *C. neoformans*, which needs DOPA as the basic building block for melanin. The pigment itself is brown in colour and fluorescent under UV light.

The pathogenic yeast, *Malassezia furfur*, produces a similar pigment to that of *C. glabrata*, and a role for this pigment in its pathogenesis seems likely. *C. glabrata* itself is an important human pathogen, second only to *C. albicans* as the cause of life-threatening candidosis. Only a few pathogenicity factors have been proposed for this fungus so far, such as adhesion factors and its relative resistance to common antifungal azole drugs. Pigment production may represent a novel pathogenicity mechanism.

To investigate the biosynthesis and biological function of pigment, we screened the transposon insertion mutant library of *C. glabrata*. We found mutants which produce no, less or even more pigment than the wild type strain. By sequencing we found corresponding genes which may be involved in the synthesis and in the regulation of pigment production.

Interestingly, pigment synthesis seems to be regulated by a multitude of factors, with the presence of tryptophan being the only absolute prerequisite. Synthesis is influenced for example by the presence of different nitrogen sources, like ammonium or other amino acids. Depending on the nitrogen source, pigmentation can be delayed or inhibited completely. Also, cell density has a strong effect on synthesis, with high cell numbers suppressing pigment production. We were able to show that cAMP is involved in at least some of these regulatory processes.

Furthermore, we could show that the pigment is able to protect the fungus from UV-light and hydrogen peroxide. This may hint toward a role in the protection against immune effector cells, which use reactive oxygen and nitrogen species to kill pathogens.

Interestingly, we found that the pigment is secreted into the surrounding medium by *C. glabrata*, where it is able to exert its effects on both the host cells and other members of the human microbial flora. For example, we could demonstrate that pigmented medium is able to suppress the yeast to hyphal transition of *C. albicans*, which plays a significant role in the pathogenesis of this fungus. This cross-species effect may in part be explained by the production of tryptophol as a by-product of pigment synthesis.

Financed in part by the “Studienstiftung des Deutschen Volkes”, the Federal Ministry of Education and Research (BMBF) and the EU ERA-NET PathoGenoMics programme: “Genomic approaches to unravel the molecular Mechanisms of pathogenicity in the human fungal pathogen *Candida glabrata* – FunPathCaGla”.

## 4 Iron Sources During Oral Infections

Group Leaders: Ricardo Sergio Couto de Almeida, Bernhard Hube

Iron is an essential element for almost all organisms, from microbes to multicellular animals. Using high affinity iron-binding molecules, higher organisms can sequester virtu-

ally all free iron, causing a natural resistance to infection known as "nutritional immunity". Therefore, pathogenic microbes are forced to utilize iron from host molecules during infection. For example, within the oral cavity, extracellular iron is mostly bound to lactoferrin found in saliva and intracellular iron is stored in association with ferritin.

Ferritin is the main intracellular storage protein for iron containing 30 % of the total iron of the human body (66 % is bound by hemoglobin). Ferritin consists of a 24-subunit protein shell of approximately 500 kDa. One ferritin molecule can contain up to 4,500 $Fe^{3+}$ ions. The quaternary structure of ferritin is dissociated at acidic pH. Under iron-limiting conditions, cytosolic ferritin is autophagocytosed and subsequently degraded within the acidic lysosomes and the iron is allocated to the cell. Outside of lysosomes, ferritin is an extremely robust and stable protein which seems to be resistant to all known microbial activities. In fact, the only microorganism that has so far been shown experimentally to exploit holoferritin as an iron source during interaction with host cells is *Neisseria meningitidis*. The bacteria can trigger degradation of cytosolic ferritin within infected epithelial cells by manipulating the cellular machinery and lysosomal activity.

*Candida albicans* is a polymorphic yeast which belongs to the normal microbial flora of human beings. The fungus lives as a harmless commensal on mucosal surfaces in healthy individuals but can cause several types of infections in predisposed patients, ranging from superficial to life threatening disease. To acquire iron, *C. albicans* possesses three known iron up-take pathways:

Up-take and usage of iron from hemoglobin. This pathway is composed of a hemoglobin receptor (Rbt5) and a heme oxygenase (Hmx1) which degrades hemoglobin to release the iron.

The siderophore receptor Sit1 can mediate the up-take of a range of siderophores (microorganism-derived iron chelators) and other iron complexes.

To use free iron from the environment, from transferrin or from other so far unknown iron sources, *C. albicans* uses the reductive iron uptake system. This system is located in the plasma membrane and consists of three activities. Firstly, insoluble extracellular ferric ($Fe^{3+}$) chelates can be reduced into soluble $Fe^{2+}$ ions by reductases. Since reduced ferrous iron generated by surface reductases can be toxic due to the spontaneous generation of free radicals, the $Fe^{2+}$ ion is subsequently oxidized to $Fe^{3+}$ and transported into the cell by a protein complex consisting of a multicopper oxidase and an iron permease.

During oral infections, *C. albicans* must be able to exploit the host iron resources. Since we observed that the ferritin concentration within oral epithelial cells was directly related to their susceptibility to damage by *C. albicans*, we hypothesized that host ferritin may be used as an iron source by this organism.

To test whether *C. albicans* can use ferritin as an iron source in vitro, we added an iron chelator (bathophenanthrolindisulphonic acid, BPS) to the medium. Under these conditions, *C. albicans* can grow on agar at physiological pH (pH 7.4) with ferritin as the sole iron source while the baker's yeast *Saccharomyces cerevisiae* cannot. A screen of mutants lacking components of each of the three acquisition systems showed that only the reductive pathway is involved in ferritin iron utilization. The mutant *Δftr1* lacking the high affinity permease Ftr1 is able to grow with free iron, hemoglobin, but not with ferritin as the sole source of iron.

Since even *S. cerevisiae* is able to grow with ferritin when the initial pH of the medium was low (pH 5.0), we reasoned that pH plays a crucial role in the release of iron from ferritin. It is known that ferritin is unstable at acidic pH and that the natural recycling of iron from ferritin occurs in the acidic environment of lysosomes. Thus, it may be possible that *C. albicans* actively lowers the pH in its proximate vicinity. In fact, *C. albicans* is only able to use ferritin as an iron source under conditions which allow acid production (glucose, but not casamino acids as a carbon source) and acidification of the surrounding environment (low concentrations of buffer at pH 7.4).

Because *C. albicans* can use ferritin as a sole source of iron, we investigated whether *C. albicans* can bind ferritin at its surface. Using

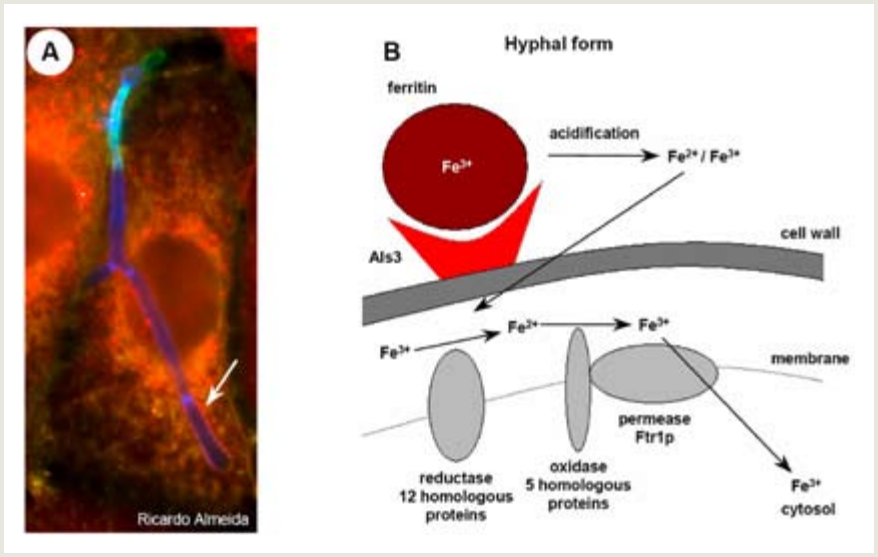


**Figure 5**
*C. albicans* can use ferritin as an iron source during oral infections.
(A) *C. albicans* wild type cells were co-incubated with oral epithelial cells and differentially stained. Red, ferritin. Blue, *C. albicans* hyphae invading oral epithelial cells. Cyan, hyphae outside. Yellow, epithelial cells membrane. The wild type hyphae, which had invaded epithelial cells, were surrounded by epithelial ferritin (white arrow).
(B) Proposed model: ferritin iron utilization by *C. albicans*

fluorescent stained anti-ferritin antibodies, we showed that the vast majority (95,8 % ± 1,53 %) of hyphae, but not yeast forms of *C. albicans* cells displayed ferritin binding. Furthermore, electron microscopy analysis showed ferritin molecules localised on the hyphal cell wall.

Because only hyphae displayed ferritin binding, mutants unable to form hyphae (*Δras1* and *Δcph1/Δefg1*) were tested in our ferritin binding assay. *Δras1* and *Δcph1/Δefg1* mutants were both completely unable to bind ferritin. To further investigate this observation, we tested the ferritin binding potential of the *Δhgc1* mutant. This mutant cannot form true hyphae but still expresses hyphal-specific genes. In fact 88,2 % ± 6,84 % of all yeast or pseudohyphae of the *Δhgc1* mutant were able to bind ferritin under laboratory hyphae-inducing conditions. These results suggest that true hyphal wild type cells and yeast or pseudohyphae of the *Δhgc1* mutant, but not the *Δras1* or *Δcph1/efg1* mutants, express a ferritin receptor.

In order to identify the predicted ferritin receptor, we incubated a wild type strain (CAI4+CIp10) (true hyphae and ferritin binding), the *Δhgc1* mutant (yeast or pseudohyphae, with ferritin binding) and *Δras1* (no hyphae and no ferritin binding) in the presence of ferritin. After 1.5 h, the RNA from all three strains was isolated, labeled and hybridized to *C. albicans* microarrays. Microarray data from four independent experiments were analyzed. We reasoned that candidate genes encoding putative ferritin receptors should be up-regulated in wild type and *Δhgc1* cells, but should be unaltered or down-regulated in the *Δras1* mutant. A total of 22 genes were identified with the expression profile indicative of a putative ferritin receptor. Three of these genes are known to encode hyphal-specific proteins which are cell surface localized as expected for a receptor protein. Consequently, these three genes were further investigated.

The three genes encoding cell surface localised and hyphae-specific proteins were *ECE1*, *HYR1* and *ALS3*. *ECE1* (**E**xtent of **C**ell **E**longation) is

a hyphal-specific gene of unknown function, the expression of which increases during elongation of the hyphal cell. The gene encodes a predicted cell membrane protein and the corresponding knockout mutant displayed no obvious phenotype. *HYR1* (**HY**phally **R**egulated) encodes a GPI-anchored protein predicted to be cell wall localized. Yet, there is no clear function identified for this protein. Finally, *ALS3* (**A**gglutinin-**L**ike **S**equence) encodes a hyphal-specific cell wall protein (GPI-anchored) which belongs to a family of adhesins (the ALS family) and plays a crucial role in epithelial and endothelial adhesion and invasion.

To show if one of these three proteins plays a role in ferritin binding, the corresponding knockout mutants were tested for ferritin binding. Both, the *Δece1* and the *Δhyr1* mutants efficiently bound ferritin at similar rates compared to the wild type. In contrast, the *Δals3* mutant completely lost its ability to bind ferritin. An *Δals3::ALS3* revertant strain reconstituted the wild type ferritin binding phenotype. This result demonstrated that Als3 plays a crucial role in ferritin binding and may indeed be the ferritin receptor.

Several lines of evidence suggest that Als3 is a specific receptor which permits binding of ferritin and iron acquisition. (1) The capacity to bind ferritin can be blocked by UV-light which suggests that binding is mediated by a protein. (2) Only hyphae, but not yeast cells bind ferritin and Als3 is known to be a hyphal specific protein. (3) A mutant lacking *ALS3* is completely unable to bind ferritin and an *Δals3::ALS3* revertant strain restores the capacity to bind ferritin. (4) The lack of *ALS3* causes a growth defect on agar plates with ferritin as the sole source. Finally, (5) S. *cerevisiae* strains expressing Als3 can bind ferritin.

Additionally, binding of ferritin to hyphal surfaces not only occurs with exogenously added purified ferritin, but also during interactions of *C. albicans* with epithelial cells. The hyphae invading epithelial cells display an accumulation of ferritin on their surface. Furthermore, the *Δals3* mutant did not show ferritin accumulation on the hyphae, while the *Δals3::ALS3* revertant strain had dense layers of ferritin on hyphal surfaces. This data is supported by the fact that both the *Δals3* and *Δftr1* mutants lost their ability to damage epithelial cells and this was not influenced by saturation of epithelial cells with ferritin as demonstrated for the wild type strain (reported above).

Taken together, our data suggest that ferritin can be used as an iron source by *C. albicans* via direct and specific binding to Als3 on the surface of hyphae. We propose that iron is then released by acidification and up-take is facilitated by the reductive pathway.

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## 5 Proteases as Virulence Factors

Group Leaders: Antje Albrecht, Lydia Schild, Bernhard Hube

Proteolytic enzymes fulfil multiple cellular functions such as protein degradation, activation of protein precursors and digestion of nutrients. Moreover, in pathogenic microorganisms, secreted proteases can act as important virulence factors. They enable pathogens to invade the host tissue or to evade the host immune system. We work on the family of secreted aspartic proteases (Saps), one major group of virulence factors which contribute to the pathogenicity of *Candida albicans*. Among the ten described Sap proteases, Sap9 and Sap10 take an exceptional position.

*SAP9* and *SAP10* code for preproenzymes with a signal peptide removed in the endoplasmic reticulum and a propeptide with Lys-Arg residues, known as proteolytic processing sites for Kex2. Like all other members of the Sap family, the mature Sap9 and Sap10 proteins possess four conserved cysteine residues and two conserved aspartate residues. Sequence comparisons revealed that *C. albicans* Sap9 and Sap10 differ from the other Sap1–8 isoenzymes not only by sequence similarity but also by multiple *N*-glycosylation sites and putative GPI anchor attachment sequences at their C termini (Eisenhaber et al., 2004), a recognized structural property of yapsins.

**Figure 6**
Oral epithelial tissue based on Reconstituted Human Epithelium (RHE) infected and damaged by invading *C. albicans* cells
(Schaller et al. (2006) *Nature Protocols* 6, 2767–2773).

To determine whether Sap9 and Sap10 are in fact GPI proteins, we used a series of constructs to express native and C-terminal truncated versions of Sap9 and Sap10 in the yeast *Pichia pastoris*. Expression of native Sap9 and Sap10 prevented secretion of these proteases. However, secretion was observed when parts of the C-terminal sequences of the GPI anchor consensus sequence were deleted and was highest when the complete consensus sequence was removed. These data suggest that the C-termini prevent secretion of Sap9 and Sap10. For cellular localization of the two proteases, we constructed Sap9- and Sap10-Gfp fusion proteins. Immunoelectron microscopy using an anti-GFP antibody revealed that both fusion proteins are located on the cell surface of *C. albicans*; Sap9-GFP was predominantly located in the cell membrane, whereas Sap10-Gfp was located in both the cell membrane and the cell wall.

SDS protein gel analysis of purified Sap9 and Sap10 proteins revealed two subunits for Sap9, with sizes of ~68 and 10 kDa and only one subunit with 60 kDa for Sap10. In contrast to other members of the Sap family, which possess a maximum of one potential *N*-glycosylation site within the sequence of the mature enzyme, Sap9 and Sap10 contain, respectively, five and eight such sites. Treatment of Sap9 and Sap10 with *N*-glycosidase F caused a clear band shift for both proteases, demonstrating that Sap9 and Sap10 are *N*-glycosylated. Thus, Sap9 and Sap10 are highly *N*-glycosylated proteases, which are GPI-anchored on the cell surface.

To determine the possible functions of the two proteases, we constructed *Δsap9* and *Δsap10* single mutants and a *Δsap9/Δsap10* double mutant. All mutant strains grew normally on media containing different carbon sources, at different pH values, on hyphal inducing media or when incubated at different temperatures. However, components that directly or indirectly target the fungal cell surface such as hy-

gromycin B, amorolfine, calcofluor, Congo red, and itraconazole caused significant growth defects for all mutants. In addition, nikkomycin inhibited growth of the *Δsap9* mutant. Observed phenotypes were at least partially restored in mutants carrying plasmid-borne Sap9 or Sap10. Together, these data suggest a role of Sap9 and Sap10 in cell surface integrity. Furthermore, all three mutants exhibited an abnormal budding phenotype, as daughter cells did not separate from mother cells and remained associated in cell chains. Because it is known that blockage of protein activities involved in cell membrane or cell wall maintenance can be compensated for by the up-regulation of proteins involved in the production of alternative structural elements, we quantified the protein and chitin levels in the cell walls of the mutants. Chitin levels of the *Δsap9* mutant were significantly increased (4.0 ± 0.9 % S.D.; *t* test, $p = 0.05$), whereas the *Δsap10* mutant showed increased levels of both protein (7.5 ± 0.6 %; *t* test, $p \leq 0.05$) and chitin (3.4 ± 0.7 %; *t* test, $p \leq 0.05$) as compared with the parental strain (protein 5.8 ± 0.7 %; chitin 1.9 ± 0.5 %).

Our results, together with previous data (Newport and Agabian, 1997), suggest that Sap9 and Sap10 and the processing serine protease Kex2 in *C. albicans*, all may be involved in the maintenance of cell surface integrity. To investigate whether Sap9, Sap10, and Kex2 have overlapping functions, we constructed *SAP9* and *SAP10* overexpression vectors, which were integrated into the single mutants (*SAP9* into *Δsap10* or *Δkex2*, respectively; *SAP10* into *Δsap9* or *Δkex2*, respectively). Phenotypic screening of these strains showed little or no overlapping function of the three proteases. Real time reverse transcription PCR analysis of the mutants revealed a 2 fold overexpression of *SAP9* in the *Δkex2* mutant, a 2 fold overexpression of *SAP9* in the *Δsap10* mutant, and a 4 fold over expression of *SAP10* in the *Δsap9* mutant, suggesting that the loss of one processing protease gene induces up regulation of the other genes.

To investigate whether dysfunctions in cell surface integrity resulting from *SAP9* or *SAP10* deletion influenced virulence of *C. albicans*, we studied the potential of *Δsap9* and *Δsap10* mutants to cause infections. Both mutants were only moderately attenuated in a mouse model of systemic infection after intravenous challenge. However, the same mutants had a significantly reduced ability to invade and damage epithelial cells in a model of oral infection based on RHE. One possible explanation for the attenuated virulence phenotype in the RHE model could be a reduced ability of the mutant strains to adhere to epithelial cells, thus resulting in reduced invasion and cell damage. This may indeed be the case for *Δsap10*, as adherence of this mutant to buccal epithelial cells was reduced. However, the ability of the *Δsap9* mutant to adhere to epithelial cells was dramatically increased as compared with the wild type, suggesting that properties other than reduced adhesion attributes were responsible for the decreased epithelial cell damage of *Δsap9*. Interestingly, the phenotype of the double mutant *Δsap9/Δsap10* showed reduced adhesion resembling the phenotype of the *Δsap10* and not the *Δsap9* single mutant. Therefore, activities of Sap9 and Sap10 are necessary for wild type adhesion properties and invasion and cell damage of oral epithelial cells.

If *SAP9* and *SAP10* play a virulence role during oral infections, both of these genes would be predicted to be expressed *in vivo by C. albicans* strains colonizing and infecting the oral cavity. To demonstrate this possibility, we analyzed *SAP9* and *SAP10* expression in total RNA samples isolated from the saliva of a large number of patients (40 symptomatic *C. albicans* infected and 29 asymptomatic *C. albicans* carriers). *SAP9* and *SAP10* transcripts were detected in 98 and 93 % of *C. albicans*-infected patients, respectively, and in 83 and 86 % of *C. albicans* carriers, respectively. This indicates that both *SAP* genes are frequently expressed both in the commensal stage and during infection, suggesting an important role for growth and/or survival in the oral cavity.

Because Sap9 and Sap10 were exposed to the extracellular space but attached to the cell membrane or cell wall, we concluded that proteolysis by Sap9 and Sap10 must take place on

the cell surface and that putative substrates of these proteases may be of either host or fungal origin. To identify possible target proteins of Sap9 and Sap10, several host proteins known to be substrates of other Saps (Naglik et al., 2003) were exposed to the proteases heterologously expressed in *Pichia pastoris*. None of the tested proteins, including serum albumin, collagen, hemoglobin, keratin, mucin, or immunoglobulins were hydrolysed in a Sap2-like manner. In contrast, several synthetic peptides containing basic or dibasic amino acid motifs were digested by Sap9 or Sap10 or by both proteases. Digests were similar to the activity of *S. cerevisiae* yapsins or Kex2 regulatory proteases with hydrolysis at KR, KK, or single (K) sites. Sap9 and Sap10 preferred cleavage after dibasic (KR, KK) or monobasic (K, R) residues, similar to yapsin 1 and 2, whereas the Kex2 protease cleaves only C-terminally to clusters of dibasic residues (Komano et al.,1999; Olsen et al., 1998; Rockwell and Fuller, 1998). In addition, Sap10 digested at sites previously unknown for yapsin-like aspartic proteases (between Phe Ser and His Asn).

Prior to further experiments, we monitored specific activity of the proteases under different reaction conditions by quantifying the cleavage of a fluorogenic peptide. Both proteases showed highest activity in a pH-range of 6 to 7. Following this, we analysed a larger set of synthetic peptides (Protease Substrate Set, JPT Peptide Technologies GmbH) to define consensus sequences for substrate processing events. The combined *in vitro* cleavage data suggest that Sap9 and Sap10 are proteases that hydrolyse polypeptides at distinct processing sites. Dibasic residues have been confirmed to be common but not exclusive motifs in Sap9 and Sap10-digested peptides. Furthermore, amino acids surrounding dibasic residues are important determinants of cleavage preference.

Interestingly, the peptide KIHNKLFGF, which is identical to an internal sequence of Sap9 (Lys149 to Phe157 from the N terminus), was processed by Sap9 between Lys and Leu154. This suggests self-processing activity of Sap9 at this site, which possibly accounts for the two subunits observed in this study and by others (Cawley et al., 2003). The N-terminal sequence of the larger β-subunit was previously shown to be Leu154 Phe Gly Phe (Cawley et al., 2003), identical to the N terminal sequence of one fragment of the digested peptide.

In summary, these data suggest that Sap9 and Sap10 are GPI-anchored proteases which target proteins necessary for both cellular processes and host-pathogen interactions.

## 6 Interaction of *Candida albicans* with Neutrophils

Group Leaders: Antje Albrecht, Bernhard Hube

*Candida albicans* commonly causes superficial mucosal infections. In immunocompromised patients however, *C. albicans* may penetrate into deeper tissue, enter the bloodstream and disseminate within the host, causing life-threatening systemic infections. The bloodstream is therefore essential for the distribution of fungal cells.

In order to elucidate how *C. albicans* responds to the challenge of a blood environment, we analysed the transcription profile of *C. albicans* cells exposed to human blood by using genomic arrays and a cDNA subtraction protocol.

We profiled *C. albicans* by inoculating cells into heparinized fresh human blood or plasma, incubating under semi-aerobic conditions at 37°C. When *C. albicans* cells enter the bloodstream, they encounter a new environment to which they rapidly adapt by expressing a distinct set of genes while down-regulating other genes. Despite the blood being a hostile environment, *C. albicans* cells remain physiologically active following exposure to blood. Within the first 10 min, the cells up-regulate the expression of the 12 % of genes that encode components of the protein synthesis machinery, thus enabling the fungus to respond to other specific demands they encounter while exposed to blood. After 30 min of being exposed to blood, 40 % of *Candida* cells begin to produce germ tubes, the initial phase of hyphal growth. This morphologic shift is accompanied by accelerated expression of hyphal growth-associated genes. However, overall growth is significantly reduced for those

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cells in blood compared to those in plasma. Many *C. albicans* cells associate with leukocytes as early as 10 min after being exposed to blood. At this time, the cells up-regulate genes associated with oxidative stress, the glyoxylate cycle, and, surprisingly, glycolysis. When phagocytic cells ingest microbes, the microbes presumably encounter a nutrient-poor environment, which is consistent with induction of the glyoxylate cycle for growth in sugar poor media and the oxidative burst of aggressive oxygen-containing molecules that results in oxidative stress. However, up-regulating both glycolysis and the glyoxylate cycle seems contradictory, leading us to suspect that we are detecting two populations of *C. albicans* cells: those that are phagocytosed and those that are not.

### *C. albicans* Cells Respond Differently to Specific Blood Components

*C. albicans* cells respond to the soluble and cellular components of human blood. Soluble components of plasma contain numerous antimicrobial activities, including factors of the complement system that can directly kill microbes and iron-binding proteins such as transferrin that sequester this essential growth factor. Many cellular components of blood, apart from the erythrocytes, have potent antimicrobial activities. For example, the granulocytes, or polymorphonuclear leukocytes, are composed of neutrophils (50–75 % of leukocytes), eosinophils (1–5 %), and basophils (0–1 %). The monocytes (2–8 % of leukocytes) are potential phagocytic cells that convert to macrophages in infected tissues. Lymphocytes (B- and T-cells) represent 20–40 % of the leukocytes and are key elements of cell-mediated and adaptive immunity. We separated blood into four fractions consisting of plasma, red cells, mononuclear cells (lymphocytes and monocytes), and granulocytes (mainly neutrophils), resuspended those three cell fractions into plasma at the same concentration as in whole blood, and then introduced *C. albicans* for 30 min before analysis by microscopy, cell viability tests, and transcriptional profiling.

More than 80 % of the *C. albicans* cells that were exposed to plasma, red cells, or mononuclear cells produced germ tubes, whereas only 4 % of the fungal cells exposed to neutrophils had germ tubes. When we tested fungal cell viability, 57 % of cells survived whole blood for 60 minutes, more than 80 % remained viable in the mononuclear cell fraction, 96 % remained viable in the red cell fraction, and 100 % remained viable in plasma. However, only 38 % survived in the neutrophil fraction. Therefore, neutrophils, but not red cells, monocytes or lymphocytes, or plasma components, block growth and kill fungal cells in blood efficiently. What accounts for more *C. albicans* cells surviving when exposed to whole blood than when exposed to neutrophils? Plasma is a strong inducer of hyphal formation, and cells immediately switch to this growth form when they contact plasma. Would it be an advantage for *C. albicans* to grow in the hyphal form in blood? Other studies indicated that factors secreted from hyphal cells inhibit killing by neutrophils; *C. albicans* cells phagocytosed by macrophages produce hyphae that penetrate membranes and kill the macrophage; hyphal cells, but not yeast cells, induce phagocytosis by endothelial cells, perhaps enabling *C. albicans* to escape the bloodstream. Thus, switching to hyphal growth may have advantages over the yeast form. However, it is not the hyphal morphology but its transcriptional program that likely accounts for hyphae-associated pathogenesis. Despite their reduced virulence, even those cells that are locked in the yeast phase retain some ability to cross the endothelial barrier of blood vessels.

### Granulocytes Govern *C. albicans* Transcriptional Response

The gene expression patterns of *C. albicans* cells exposed to whole blood or to neutrophils are similar. Thus, neutrophils apparently govern the transcriptional response of *C. albicans* in human blood, whereas the red cells, mononuclear cells, and plasma appear to have only a minor influence. Gene expression profiles reflect the physiological condition of cells and the microenvironment to which they are exposed. For example, when cells were exposed to red cells, mononuclear cells, or plasma, the *C. albicans* genes associated with protein synthesis, glycolysis and hyphal formation are up-regulated. In contrast, when cells were

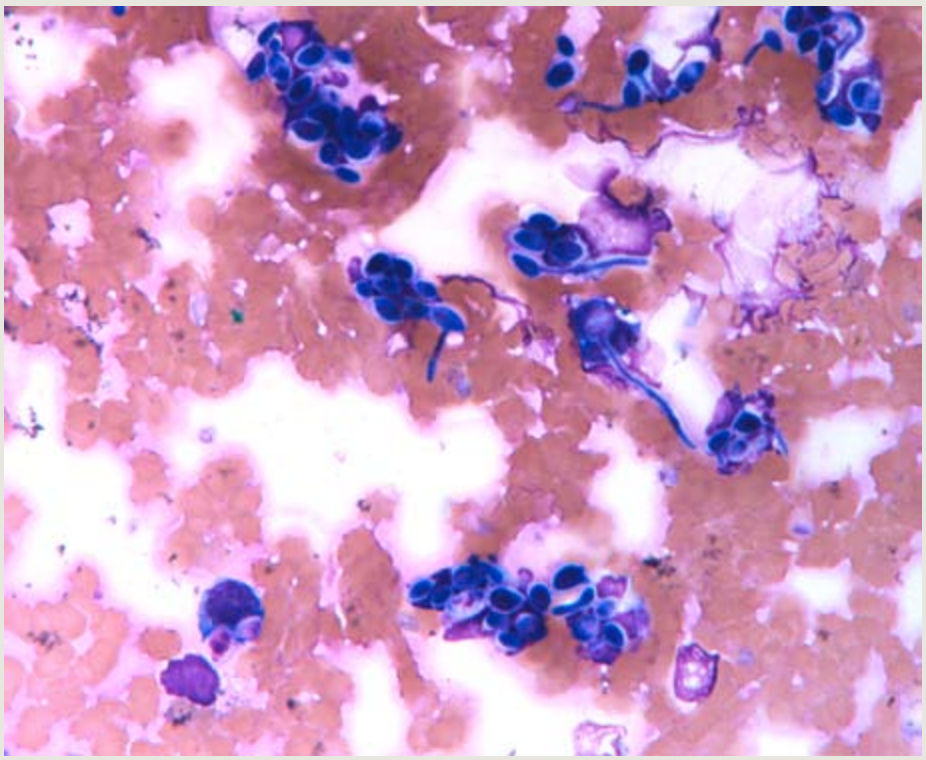

**Figure 7**
*Candida albicans* cells exposed to human blood are able to survive the attack of phagocytes.

exposed to neutrophils, genes associated with detoxification of reactive oxygen species, the glyoxylate cycle and amino acid metabolism. Thus, the transcript profile of *C. albicans* in blood reveals two populations of cells, some of which are in contact with neutrophils while the rest are not. How do neutrophils arrest growth and induce oxidative stress? Phagocytosis may lead to antifungal components such as lactoferrin, elastase, defensins, and reactive oxygen species being released into the phagolysosome. Alternatively, these components may be secreted into the extracellular space. Although phagocytosis would seem to explain the starvation response, only 38% of *C. albicans* cells are phagocytosed. However, exposure to neutrophils strongly upregulates genes linked to oxidative stress, including genes encoding catalases and superoxide dismutases. These enzymes eliminate endogenous reactive oxygen species, generated during respiration. In pathogenic microbes, they may have an additional function in detoxifying reactive oxygen species caused by the oxidative burst of phagocytic cells.

*C. albicans* contains as many as six genes encoding superoxide dismutases (*SOD1-SOD6*), three of which have C-terminal sequences that are typical of proteins attached to cell membranes or the cell wall. Why produce superoxide dismutases that face the extracellular space? Possibly these enzymes detoxify reactive oxygen species released by phagocytic cells that the fungal cells encounter.

Our finding that the surface-associated Sod5 is induced when yeast cells contact or are phagocytosed by neutrophils supports this idea.

Transcript profiling shows that neutrophils dominate the host response by confronting *C. albicans* cells with a hostile environment. To survive, the fungus induces genes involved in amino acid pathways, nitrogen metabolism, the glyoxylate cycle and oxidative stress response. Thus, microbes can act as biosensors, and transcript profiling is a way to read their output. In this case, neutrophils are key to the

host defence against invading *C. albicans* cells. However, the fungus induces a large number of genes to counteract the neutrophils.

### The Early Transcriptional Response of Human Granulocytes to Infection with *Candida albicans* is not Essential for Killing but Reflects Cellular Communications

To compare the transcriptional response of leukocytes exposed to *C. albicans*, we also investigated the expression of key cytokine genes in polymorphonuclear and mononuclear leukocytes after incubation with *C. albicans* for 1 h. Isolated mononuclear cells expressed high levels of genes encoding proinflammatory signalling molecules, whereas neutrophils exhibited much lower levels, similar to those observed in whole blood. The global transcriptional profile of neutrophils was examined by using an immunology-biased human microarray to determine whether different morphological forms, or the viability of *C. albicans* altered the transcriptome. Hyphal cells appeared to have the broadest effect, although the most strongly induced genes were regulated independently of morphology or viability. These genes were involved in proinflammatory cell-cell signalling, cell signal transduction and cell growth. Generally, genes encoding known components of neutrophil granules showed no upregulation at this time point; however, lactoferrin, a well known candidacidal peptide, was secreted by neutrophils. Addition of inhibitors of RNA or protein de novo synthesis did not influence the killing activity within 30 min. These results support the general notion that neutrophils do not require active gene transcription to mount an immediate and direct attack against microbes. However, neutrophils exposed to *C. albicans* express genes involved in communication with other immune cells.

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## 7 Experimental Infections

Group Leader: Ilse Jacobsen

The use of suitable infection models for the analysis of virulence and pathogenicity is a fundamental element in the study of pathogenic fungi and other microbial pathogens. Murine models are the gold standard for testing fungal pathogenesis to date. However, whenever possible, alternative *in vitro*, *ex vivo* or *in vivo* infection models are used. We routinely use the following infection models:

### Invasive Aspergillosis in Mice

Invasive aspergillosis is a life-threatening disease in immunocompromised patients. In order to elucidate mechanisms of both the host and the pathogen that contribute to pathogenicity, murine models have been developed. We commonly use these models to study the role of individual fungal genes during infection. Therefore mice are infected with defined fungal knockout mutants. Mice are only susceptible to *Aspergillus* infections if immunocompromised. We use immunosuppresion by both cyclophosphamide (panleukopenic) and by cortisone (reduced phagocyte function) to mimic different kinds of immunosuppression that put human patients at risk of invasive Aspergillosis. The animals are infected intranasally under general anaesthesia. Before fungal mutants are tested *in vivo*, selected *in vitro* and *ex vivo* models are used to gain some insight into the phenotypic characteristics of these mutants. This allows us to choose the right model to test attenuation of mutants.

### Infections with *Candida albicans*

*C. albicans* infections can manifest at different body sites and range from inconvenient but comparably harmless local infections to severe and life-threatening disseminated candidosis. To address these different manifestations, different murine models have been developed. To study invasion properties of *C. albicans* mutants, which were demonstrated to have reduced in invasion *in vitro*, we use intraperitoneal infections. This model causes little distress to the animals and is therefore a

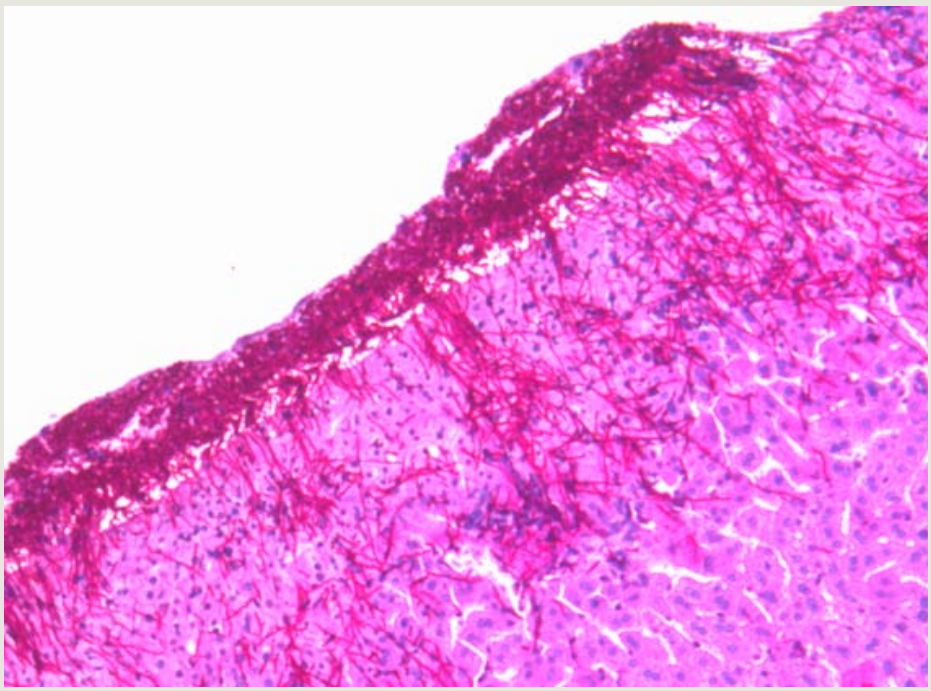

**Figure 8**
*Candida albicans* invading the liver 24 hours after intraperitoneal infection. PAS-stain.

valuable substitute for mortality studies. Since the pathogenesis of disseminated candidosis is very complex, effects of some fungal mutations can only be studied in survival-based models, such as intravenous infection of mice. To minimize the suffering of animals in these studies, infected animals are closely monitored. Clinical symptoms, weight loss and drop in body surface temperature are used as quantitative parameters for humane endpoints.

### Systemic *Candida glabrata* Infections

Disseminated infections with *C. glabrata* are less common than those with *C. albicans*; however, due to the higher resistance of C. glabrata to azoles, they are more difficult to treat. Furthermore, the pathogenesis of *C. glabrata* infections is poorly understood. Mammalian infection models are crucial for understanding the pathogenesis of *C. glabrata*. However, no standardized infection model for *C. glabrata* has yet been described. The murine *C. glabrata* models described in the literature vary greatly in respect to mice strains, infectious dose and immunosuppression. The common feature of all literature reports is that *C. glabrata* does not cause clinical disease in wild type mice. Analysis of infection experiments therefore relies on quantitative reisolation and histology. We are currently establishing a protocol for systemic murine *C. glabrata* infection as part of the work package of the ERA-NET PathoGenoMics Program: "Genomic Approaches to unravel the molecular basis of pathogenicity in the Human Fungal Pathogen *Candida glabrata* – FunPathCaGla". Initial experiments demonstrated that following systemic infection, *C. glabrata* colonizes all organs investigated, including the brain. Significant fungal burdens are maintained for weeks after infection. Although immunosuppression leads to higher fungal burdens, it is not a necessary prerequisite for successful infection.

### Refinement of *in vivo* Infection Models

Animal experiments entail careful ethical considerations by the involved researchers. It is mandatory to design experiments such that distress for the animals is minimized. One way to improve the well-being of laboratory rodents is to provide an appropriate environment that is in accord with the animals behavioural needs without influencing the results of the experiment. We use autoclavable mouse houses to provide mice with a nest-like structure for resting and an additional level in the cage for explorative behaviour. We found that this simple addition greatly increases differential behaviour and well-being of our animals without influencing experiments results.

In survival experiments humane endpoints are an important way to minimise suffering of animals. In addition to the general clinical condition of animals, we use body weight and non-invasive measurements of body temperature to predict imminent death of an animal. In this situation, animals are then euthanized. This approach not only decreases distress for the animals but also allows sampling of body fluids and tissues for further analysis.

### Histology

Survival of infected animals provides information about the virulence of the strains under investigation, but not about pathogenicity mechanisms. Therefore, histological analysis of organ samples are used to increase the informative value of infection experiments. Histology allows evaluation of fungal morphology, characteristics of tissue damage and the immune response of the host. A collaboration with the Fritz-Lipmann-Institute (FLI) at the Beutenberg Campus was established to process tissue samples for histopathology, which are then analysed by the group.

### Alternative Infection Models

Several *in vitro* and *ex vivo* alternative models have been established in our department and are used routinely, for example models based on Reconstituted Human Epithelium (RHE) and primary human blood cells. These models are extremely useful for studying certain aspects of pathogenesis but do not reflect the complex environment which fungi encounter during mammalian infection.

An alternative *in vivo* model is the embryonated egg – this provides a complex environment, including phagocytic cells, to study fungal host-pathogen interactions, but is of a lower developmental stage than adult mice.

Dr. Härtl, previous leader of the Drug Testing Group, has established an egg model (*in ovo*) for testing the efficacy of antifungal drugs against candidosis. We are currently establishing this model for *Aspergillus* infections, aiming at a screening tool to investigate the virulence of deletion mutants. We found that in ovo infection with *A. fumigatus* is dose-dependent and highly reproducible. Comparison of *A. fumigatus* mutant strains in ovo and in mice showed good correlation. Therefore, in ovo infections appear to be a valuable alternative model to screen mutant strains and reduce the number of laboratory rodents used in infection experiments.

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Teilprojekt: Identification and characterisation of virulence associated genes during oral infections with *Candida albicans*
Bernhard Hube

Deutsche Forschungsgemeinschaft
Schwerpunktprogramm 1160: Kolonisation und Infektion durch humanpathogene Pilze
Teilprojekt: Pathogenitätsfaktoren von *Malassezia furfur*: Genetische Regulation Tryptophan-abhängiger Sekundärmetabolite und ihre Wirkung auf immunkompetente und strukturelle Zellen der menschlichen Haut
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Bundesministerium für Bildung und Forschung and European Union
ERA-NET PathoGenoMics Programm
Projekt: FunPathCaGla – Genomic Approaches to unravel the molecular basis of pathogenicity in the Human Fungal Pathogen *Candida glabrata*
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European Union
6. Forschungsrahmenprogramm der EU, Marie Curie Research Training Networks
GALAR FUNGAIL 2 – Interaction of fungal pathogens with host cells: a post-genomic approach
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Albert Härtl

Deutscher Akademischer Austauschdienst (DAAD) / CAPES Coordenação de Aperfeiçoamento de Pessoal de Nível Superior
*Candida albicans* oral infections and the role of genes involved in iron metabolism
Ricardo S. Couto de Almeida

Studienstiftung des Deutschen Volkes
Pigmentsynthese bei humanpathogenen Hefen
Sascha Brunke

National Institutes of Health (NIH)
Development of *in vitro* models of human oral mucosa relevant to AIDS and mucosal infections
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(HKI authors in bold)


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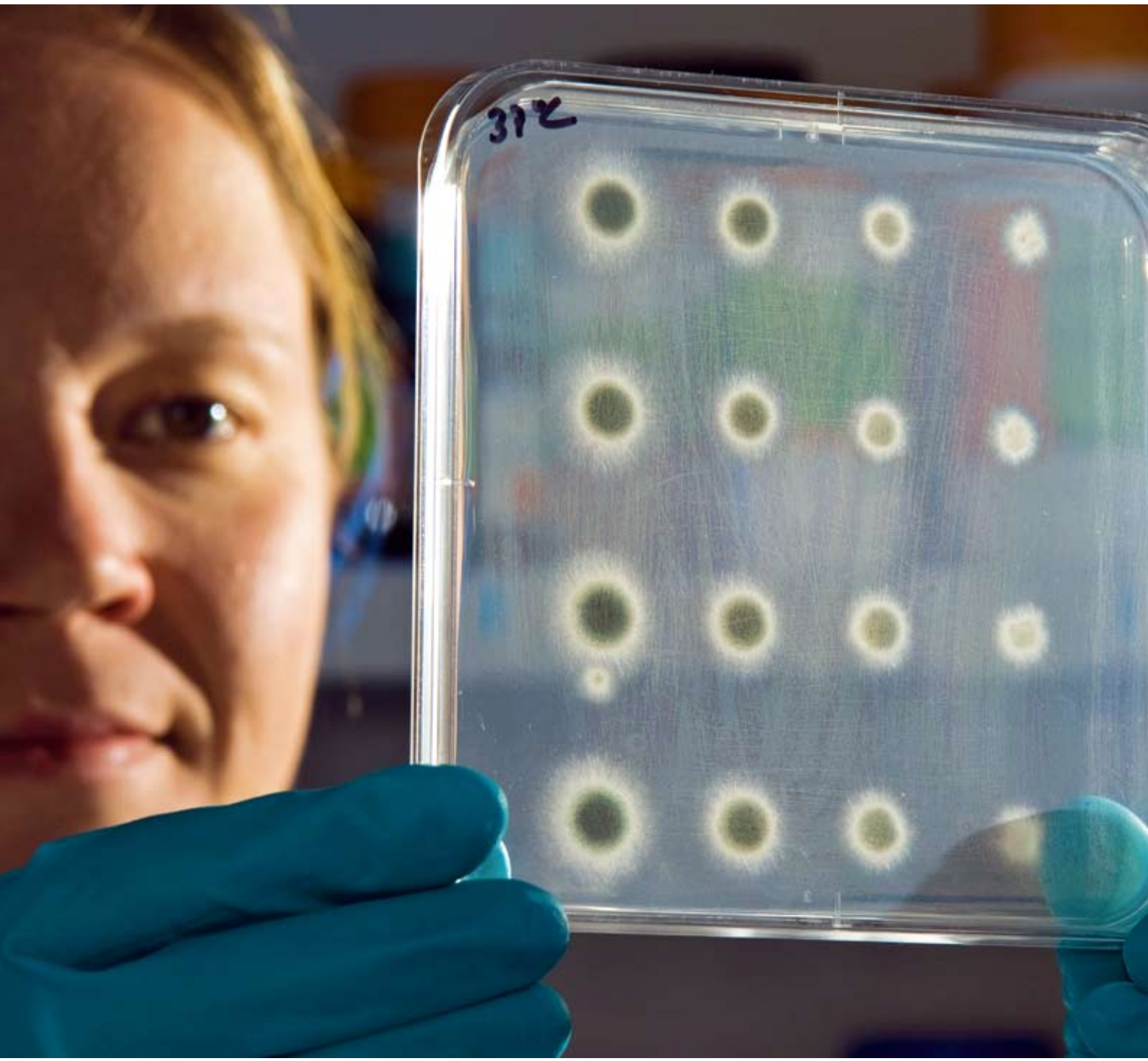

# Department of Molecular and Applied Microbiology

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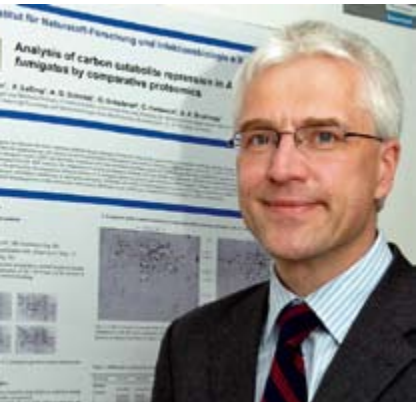

The Department of Molecular and Applied Microbiology is devoted to research in the two main areas of the Leibniz Institute of Natural Product Research and Infection Biology (HKI).

### 1. Pathobiology of *Aspergillus fumigatus*

During the past 20 years, the incidence of fungal infections in humans has risen considerably. This increase in infections is associated with excessive morbidity and mortality and is directly related to increasing patient populations at risk for the development of serious fungal infections, which includes individuals undergoing solid-organ transplantation, blood and marrow transplantation, major surgery, and those with AIDS, neoplastic disease, immunosuppressive therapy, advanced age, and premature birth. Fungal infections are categorised in two groups: topical and systemic infections. Topical fungal infections affect body surfaces and can be chronic. Systemic fungal infections can occur in an organ or in the whole body and are transferred via the bloodstream. Compared to other microbial infections, systemic fungal infections are characterised by lower frequencies but generally high mortality rates (30-100 %). The most common causes of systemic infections are *Candida* spp., in particular *C. albicans*, and *Aspergillus* spp., mainly *A. fumigatus*.

***Aspergillus fumigatus*** has become the most important airborne fungal pathogen of humans. Diseases caused by *A. fumigatus* can be divided into three categories: allergic reactions

## INTRODUCTION | EINLEITUNG

**Head:**
**Prof. Dr. Axel A. Brakhage**

Die Abteilung Molekulare und Angewandte Mikrobiologie befasst sich mit den beiden Forschungsschwerpunkten des HKI, der Naturstoff-Forschung und Infektionsbiologie.

### 1. Pathobiologie von *Aspergillus fumigatus*

In den letzten 20 Jahren ist ein beachtlicher Anstieg an Pilzinfektionen beim Menschen zu beobachten. Dieser Anstieg und die damit einhergehende erhöhte Letalität ist im wesentlichen auf eine wachsende Zahl an Risikopatienten zurückzuführen, die auf Grund drastischer medizinischer Eingriffe oder bestimmter Vorerkrankungen besonders empfänglich für Pilzinfektionen mit einem schwerwiegenden Verlauf sind. Zu den prädisponierenden Faktoren gehören insbesondere Organtransplantationen, die Übertragung von Blut und Knochenmark und andere schwere chirurgische Eingriffe mit anschließender immunsuppressiver Therapie, AIDS-Erkrankungen, Tumoren, aber auch Frühgeburt oder ein fortgeschrittenes Alter. Pilzinfektionen treten als topische oder systemische Erkrankungen in Erscheinung. Topische fungale Infekte betreffen in erster Linie Körperoberflächen, sie können chronisch verlaufen. Systemische Infektionen betreffen einzelne Organe oder den Gesamtorganismus. Sie werden durch den Blutstrom im Körper verbreitet. Verglichen mit anderen mikrobiell bedingten Infektionen treten systemische Pilzinfektionen zwar seltener auf, sie sind jedoch durch eine sehr hohe Letalität gekennzeichnet, die je nach untersuchter Fallgruppe zwischen 30 und 90 % liegt. Die wichtigsten Erreger systemischer Pilzinfektionen sind Hefen der Gattung *Candida*, insbesondere *Candida albicans*, sowie Hyphenpilze der Gattung *Aspergillus*, darunter hauptsächlich *A. fumigatus*.

***Aspergillus fumigatus*** ist heute das wichtigste über die Luft verbreitete pilzliche

and colonisation with restricted invasiveness are observed in immunocompetent individuals while systemic infections with high mortality rates occur in immunocompromised patients. Specific diagnostics are still limited as are the possibilities of therapeutic intervention, leading to the disappointing fact that invasive aspergillosis is still associated with a high mortality rate that ranges from 30% to 90%. For example, during the past 15 years, invasive aspergillosis has become the main cause of death in patients with acute leukemia and liver transplantation. A recent retrospective study on the risk and outcome of *Aspergillus* infections from 251 lung transplant recipients led to the finding that *Aspergillus* was isolated from 86 (33%) cases, which involved colonisation (n=50), tracheobronchial lesions (n=17) or invasive aspergillosis (n=19). Also, a significant association was found between acute rejection and the time at which fungal infections was diagnosed. The mortality rate for invasive infections was 78%.

In recent years considerable progress has been made in understanding the genetics of *A. fumigatus* and molecular techniques for the manipulation of the fungus have been developed. Molecular genetics offers not only approaches for the detailed characterisation of gene products that appear to be key components of the infection process but also selection strategies that combine classical genetics and molecular biology to identify virulence determinants of *A. fumigatus*. Furthermore, the genome of *A. fumigatus* has been sequenced. This knowledge provides an excellent opportunity to ana-

Pathogen. Die durch *A. fumigatus* ausgelösten Krankheiten gliedern sich in drei Gruppen: Während allergische Reaktionen und Infekte mit schwacher Invasivität bei Personen mit intakter Immunabwehr auftreten, kommt es vor allem bei immunkompromittierten Patienten zu systemischen Verläufen. Die spezifische Diagnose ist ebenso erschwert wie die Möglichkeiten einer Therapie, so dass die invasive Aspergillose nach wie vor durch eine sehr hohe Letalitätsrate gekennzeichnet ist. In den zurückliegenden 15 Jahren entwickelte sich diese Infektion zur Haupt-Todesursache bei Patienten, die an akuter Leukämie leiden oder eine Lebertransplantation erhielten. Eine aktuelle, retrospektive Studie zeigt, dass von 251 Empfängern eines Lungentransplantates 86 Patienten (33%) mit *Aspergillus* infiziert waren. Die Infektionsstadien reichten dabei von Kolonisierung (n=50) über tracheobronchiale Läsionen (n=17) bis hin zur invasiven Aspergillose (n=19). Weiterhin wurde eine signifikante Assoziation zwischen einer akuten Abstoßungsreaktion und der Diagnose pilzlicher Infektionen gefunden. Die Letalität betrug dabei für die invasiven Infektionen 78% (Sole et al., 2005; *Clin Microbiol Infect* 11, 359-365).

In den zurückliegenden Jahren wurden bedeutende Fortschritte im Verständnis der Genetik von *A. fumigatus* erzielt. Wichtige Methoden für die gentechnische Manipulation des Pilzes wurden entwickelt. Die Molekulargenetik eröffnet dabei einerseits Möglichkeiten zur Charakterisierung von Genprodukten, die als Schlüsselkomponenten am Infektionsprozess beteiligt sind, andererseits verfügen wir damit über Selektionsstrategien, die eine Identifikation von Virulenzdeterminanten gestatten. Die inzwischen erfolgte Sequenzierung des kompletten Genoms von *A. fumigatus* erlaubt zudem in hervorragender Weise, pilzliche Infektionsmechanismen in ihrer gesamten Breite zu studieren.

lyse fungal infection mechanisms in a broad sense.

Research at the Department covers all relevant aspects of *A. fumigatus* to elucidate the pathobiology of *A. fumigatus*. Research includes the areas of signal transduction, elucidation of unusual pathways like the biosynthesis of melanins, improvement of genetic tools, genomics, proteomics, transcriptomics, pathogen/host (immune effector cells) interaction. Furthermore an animal model is available. The Brakhage group has identified the first virulence determinant of *A. fumigatus* which is represented by the *pksP* gene involved in the biosynthesis of the conidial pigment. Based on results obtained in the Department, the identified proteins involved in virulence will be evaluated as target proteins for antifungal drugs.

## 2. Regulation of fungal secondary metabolism genes and activation of silent gene clusters

Fungi produce numerous of secondary metabolites. Some of these compounds are used as antibiotics such as the β-lactam antibiotics penicillin and cephalosporin, or as immunosuppressants like cyclosporin. Others have been proposed to be important for virulence e.g. of the human-pathogenic fungus *Aspergillus fumigatus*. Besides the identification and characterisation of novel microorganisms producing secondary metabolites, recent research at the Department has aimed at elucidating the molecular regulation of the biosyntheses of secondary metabolites, i.e., to answer questions as under which physiological conditions are gene clusters expressed, what kind of reg-

Die Mitarbeiter der Abteilung Molekulare und Angewandte Mikrobiologie beschäftigen sich mit allen Aspekten der Pathobiologie von *Aspergillus fumigatus*. Die Forschungsarbeiten beinhalten die Gebiete Signaltransduktion, spezielle Stoffwechselwege wie Melaninbiosynthesen, die Verbesserung gentechnischer Analysemethoden, Genomik, Proteomik, Transkriptomik sowie das Studium von Pathogen/Wirt-Wechselbeziehungen. Für Infektionsstudien ist ein Tiermodell verfügbar. Der Gruppe um Axel Brakhage gelang es erstmals, eine Virulenzdeterminante von *A. fumigatus* zu identifizieren. Es handelt sich dabei um das Produkt des *pksP*-Gens, das an der Biosynthese eines Konidienpigments beteiligt ist. Die auf diese Weise identifizierten Proteine werden als potentielle Targets für neue antifungale Wirkstoffe intensiv untersucht.

## 2. Regulation pilzlicher Sekundärmetabolismusgene und Aktivierung stiller Gencluster

Pilze produzieren eine Vielzahl sekundärer Stoffwechselprodukte. Einige dieser Substanzen werden als Antibiotika genutzt, wie zum Beispiel die β–Lactame Penicillin und Cephalosporin oder Immunsuppressiva wie Cyclosporin. Andere Metabolite scheinen für die Virulenz humanpathogener Pilze bedeutsam zu sein. Neben der Identifizierung und Charakterisierung neuer Wirkstoffbildner befassen sich einige Projekte der Abteilung mit der Aufklärung molekularer Mechanismen der Regulation der Sekundärstoff-Biosynthese. So wird beispielsweise der Frage nachgegangen, unter welchen physiologischen Bedingungen bestimmte Gencluster exprimiert werden und welche regulatorischen Gene daran beteiligt sind. Genaue Kenntnisse der molekularen

ulatory genes are involved. The knowledge of the expression level of biosynthesis genes is of great importance for the production of secondary metabolites. Moreover, the identification of regulatory genes and circuits will help to elucidate both the physiological meaning of these compounds for the producing fungus and the extra- and intracellular signals controlling the biosyntheses of secondary metabolites in fungi. Furthermore, in close collaboration with the Department of Biomolecular Chemistry and the Bio Pilot Plant, we have been working on "genome mining" strategies which, together with novel gene activation strategies, allow to activate silent gene clusters of fungi. By applying these technologies, novel compounds have already been isolated. "Genome mining" combined with activation of silent biosynthesis gene clusters is an emerging area of natural product discovery and represents a new way of drug discovery.

### 3. Systems Biology / Bioinformatics

Because of the amount of data generated in the different projects, a Systems Biology approach has been integrated in the Department's effort to fully understand both infectious processes and the physiology of secondary metabolites. Therefore, *in vivo*, *in vitro* and *in silico* approaches have been combined to discover the structure and dynamics of biological systems by process data analysis and modelling, as well as to control or optimise experiments (model based experimental design), biotechnical product formation and medical processes in diagnostics and therapy.

Regulation (Transkriptionsfaktoren, Regulons) bestimmter Biosynthesegene sind entscheidend für die Produktion sekundärer Naturstoffe. Die Aufklärung regulatorischer Prinzipien ermöglicht außerdem ein besseres Verständnis der Funktion und Evolution von Naturstoffen sowie des intra- und extrazellulären Signalaustausches bei Pilzen zu erlangen. Mit Hilfe von neuartigen „Genome mining"-Strategien werden in Kooperation mit der Abteilung Biomolekulare Chemie und dem Biotechnikum stille Gencluster von Pilzen aktiviert und neue Wirkstoffe charakterisiert. Insgesamt handelt es sich dabei um ein spannendes, sich schnell entwickelndes Wissenschaftsfeld zur Identifizierung neuer Wirkstoffe.

### 3. Systembiologie / Bioinformatik

Mit der großen Menge experimenteller Daten, die in verschiedenen Forschungsprojekten der Abteilung generiert werden, wurde ein systembiologischer Ansatz möglich. Er dient dem Ziel, Infektionsmechanismen und die Physiologie der Synthese sekundärer Stoffwechselprodukte möglichst umfassend zu verstehen. Daten aus *in vivo*-, *in vitro*- und *in silico*-Versuchen werden in der Forschungsgruppe Systembiologie gemeinsam verarbeitet, um die Struktur und Dynamik komplexer biologischer Systeme verstehen zu lernen und Experimente besser zur planen (modellbasiertes Experimente-Design). Eine ausgefeilte Prozessdatenanalyse und verschiedenste Modellierungstools gestatten eine Optimierung der biotechnologischen Produktausbeute und führen zu einem besseren Verständnis biomedizinischer Prozesse. Eine öffentlich zugängliche, umfassende Datenbank wird zur Zeit etabliert (www.omnifung.hki-jena.de).

## 1 Proteomics of *Aspergillus fumigatus* and *Aspergillus nidulans*

Group Leaders: Olaf Kniemeyer, Axel A. Brakhage

### The Oxidative Stress Response of *Aspergillus fumigatus*-characterisation by Molecular Biology and Proteomics

Billion years ago, the emergence of first oxygen-producing phototrophic bacteria led to a conversion of the earth's atmosphere from an anoxic to an oxic state. The appearance of oxygen was a prerequisite for the evolution of an efficient energy metabolism based on the reduction of atmospheric oxygen to water. This process came along with the formation of reactive oxygen intermediates (ROI) in the cell's power sources, the mitochondria. ROI affect many cellular functions by damaging DNA, proteins and lipids. Such oxidative damage also plays a role in many human diseases, such as cancer, arthritis and other autoimmune diseases. On the other hand, ROI have also a protective function against pathogenic microorganisms during host defence and have a signalling role in many biological systems as well.

Phagocytic cells of the immune system such as macrophages and neutrophils release ROI. The secretion of ROI has been suggested to be involved in the killing of fungal pathogens, such as the filamentous fungus *Aspergillus fumigatus*. The mold *A. fumigatus* has become the most important airborne fungal pathogen of humans. *A. fumigatus* can cause severe, systemic infections with high mortality rates in immunocompromised individuals. The number of cases of invasive aspergillosis has increased due to the rising number of solid organ and bone marrow transplantations and high dose chemotherapy for the treatment of cancer. Hence, there is a strong need to gain a better understanding of the pathogenicity mechanisms of *A. fumigatus*. During the infection process *A. fumigatus* has to cope with dramatic changes of the environmental conditions. Since *A. fumigatus* is a predominant fungal pathogen the question arises: Does *A. fumigatus* possess better adaptation mechanisms in comparison to other filamentous fungi? For instance, does this fungus tolerate ROI better in comparison with other fungi? To address this question, we analysed the proteome of *A. fumigatus* grown under oxidative stress. A proteome of an organism is defined as the "**prote**in complement of the gen**ome**". Proteomic studies are often represented by quantitative 2D-polyacrylamide gel electrophoresis (2D-PAGE). This technique allows the separation of up to 1000 proteins. By applying 2D-PAGE the overall changes of the protein complement of an organism after stress induction is feasible.

### Study of the Oxidative Stress Response

Our aim was to characterise the defence response of *A. fumigatus* to oxidative stress. For this purpose, *A. fumigatus* was incubated with the reactive oxygen intermediate hydrogen peroxide for 45 minutes. In a control, the fungus was grown under non-stressed conditions. Fungal proteins were extracted and separated by 2D-PAGE. By comparing the protein patterns of stressed fungal mycelium in comparison to non-stressed mycelium, many proteins were found which showed an altered expression. Primarily affected were proteins involved in the heat shock response, protein translation, central metabolism, amino acid and sugar biosynthesis.

### Study of a Global Transcriptional Regulator for Defence Against Reactive Oxygen Intermediates

Several genes encoding the proteins, which were upregulated under ROI stress, are known to be regulated by a global transcriptional regulator in baker's yeast named Yap1p. A similar gene was also found in the genome of *A. fumigatus* by genome database search. The corresponding gene in *A. fumigatus* was designated

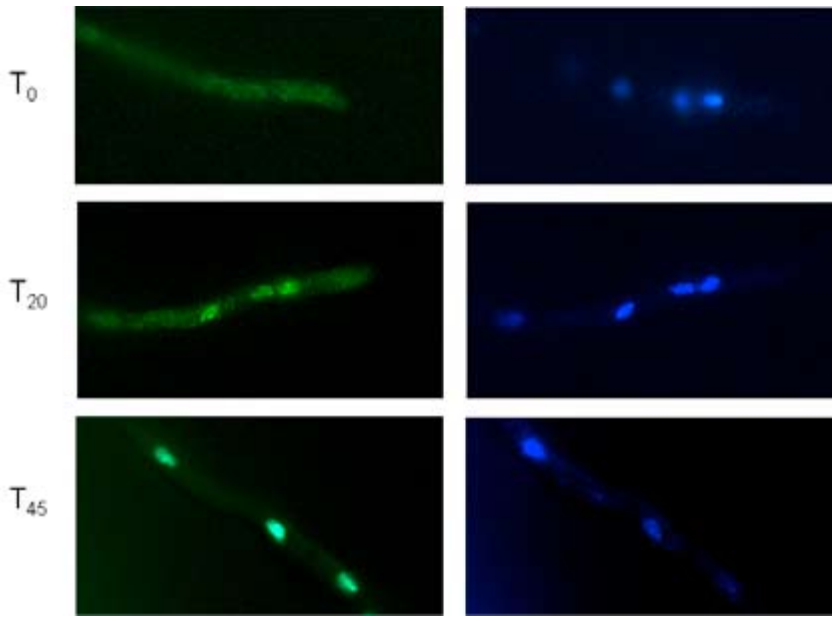


**Figure 1**
Oxidative stress-regulated localization of the global regulator AfYap1. (Left column) The fluorescence microscopy picture shows that the protein AfYap1 (green) accumulates in the nucleus after exposure to hydrogen peroxide. (Right panel) Nuclei were stained with DAPI.
This dye interacts with DNA and is often used as a nucleus-specific stain.

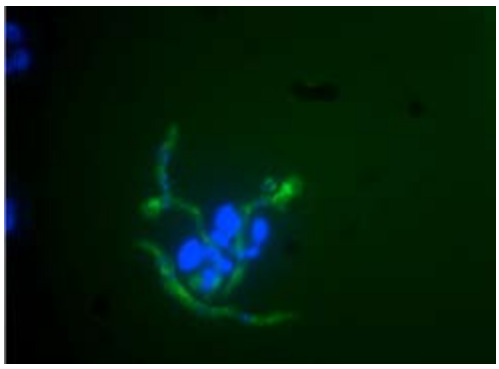

**Figure 2**
Granulocytes (blue) attacking a hyphae (green) of *Aspergillus fumigatus*.

*Afyap1*. It showed high similarity to the homologous gene in baker's yeast. To investigate the role of *Afyap1* in the oxidative stress response in *A. fumigatus* we deleted this gene in a wild-type strain of *A. fumigatus*. The generated deletion mutant, which lost its functional *Afyap1* regulator, showed a significant increase in sensitivity to ROI, such as superoxide radicals and hydrogen peroxide. Furthermore, *Afyap1* accumulated in the nucleus after exposure to hydrogen peroxide. This was shown by fusing the *Afyap1* to a green fluorescence protein. By applying this technique the cellular localisation of the protein can be observed under the microscope (Fig. 1).

### Investigation of the Targets of *Afyap1*

To elucidate possible targets of the global regulator AfYap1, we examined the effect of Afyap1 deletion at the protein level. For this purpose, protein extracts of the wild type and the Afyap1 deletion strain challenged with hydrogen peroxide were compared by 2D-gel electrophoresis. Putative targets of the regulator Afyap1 were expected to show a down regulation: the missing Afyap1-regulator would lead to a failure of gene activation of Afyap1-regulated genes. Several proteins displayed a decrease in intensity in the mutant strain and represented putative Afyap1 targets. Among the down-regulated proteins were enzymes know to be involved in the decomposition of reactive oxygen intermediates, such as catalases, a thioredoxin peroxidase etc. In addition, we also found new, formerly unknown targets, e. g, an enzyme called *p*-nitroreductase

### Investigation of the Virulence of *Afyap1*

To assess the role of AfYap1 in pathogenesis, the corresponding deletion mutant was tested in an animal infection model of invasive aspergillosis. Groups of mice were immunosuppressed and infected with conidia of the wild type and the mutant strain. If one assumed, that resistance to ROI is an important virulence factor for a pathogenic fungus, an

attenuation in virulence of the mutant strain would be expected. Surprisingly, the wild type and the Afyap1 deletion mutant lead to the same mortality rate.

To confirm these data, we also determined the killing rate of *A. fumigatus* germ tubes (freshly germed spores) during confrontation with neutrophilic granulocytes (Fig. 2). These aforementioned cells of the innate immune system are the most abundant type of white blood cells. They are highly motile and quickly congregate at a site of infection. They can internalise and kill spores, but they are also able to attack hyphae by secreting toxic compounds. Although the deletion mutant of Afyap1 showed a high sensitivity to ROI, the killing rate of this strain in comparison to the wild-type strain was the same. These data challenge the idea that the immune system, in particular granulocytes, kills *A. fumigatus* during the infection by the production of ROI. Consequently, killing mechanisms other than the production of ROI by the host appear to be more relevant. In future experiments we will elucidate these factors with a focus on non-ROI-mediated killing. A better understanding of the killing mechanism of *A. fumigatus* in the host could also help to render more precisely patient groups at risk for invasive aspergillosis and to improve antifungal therapy.

## 2 Virulence of *Aspergillus fumigatus*

Group Leaders: Thorsten Heinekamp, Axel A. Brakhage

### G Protein-coupled Receptors and Signal Transduction

The saprophytic fungus *Aspergillus fumigatus* colonises diverse habitats all over the world. Over the last decades it became the most important air-borne fungal pathogen, causing severe disseminating infections with invasive growth in immunocompromised patients. Sensing a wide variety of stimuli is essential for the fungus to adapt to different environmental conditions.

To date, one of the most important questions is still unanswered: what are the external signals and the corresponding proteins sensing those ligands or stimuli which enable the fungus to adapt its versatile metabolism to such a wide variety of ecological niches? In a first approach, two genes encoding putative G protein-coupled receptors, *gprC* and *gprD*, designated as carbon source-sensing receptors, were deleted in *A. fumigatus*. The physiological characterisation of the mutants revealed altered growth on solid media. However, various growth conditions, which included the use of different carbon- and nitrogen-sources, did not restore the defect of the mutants. The function of the putative GPCRs was further investigated by analysing fluorescent protein-fusions *in vivo* by confocal microscopy. Virulence of the mutant strains, as tested in a low-dose murine infection model, was attenuated.

In previous studies, characterisation of several elements of the cAMP signal transduction cascade indicated that this pathway contributes to the pathogenicity of *A. fumigatus*. A central component of the cAMP signaling pathway is the protein kinase A (PKA), which regulates cellular processes by phosphorylation of specific target proteins in response to changing intracellular cAMP levels. We generated and analysed *A. fumigatus* mutants which expressed the gene encoding the catalytic subunit of PKA, *pkaC1*, under control of an inducible promoter. Strains overexpressing *pkaC1* showed high PKA activity, reduced growth, sporulation deficiency and formation of a dark pigment in the mycelium. This indicates that cAMP/PKA signaling is involved in the regulation of important processes like growth, asexual reproduction and biosynthesis of secondary metabolites. Furthermore, elevated PKA activity due to deletion of the gene *pkaR* encoding the regulatory subunit of PKA, led to increased expression of the *pksP* gene. The polyketide synthase PksP is an essential enzyme for production of dihydroxynaphthalene (DHN) melanin in *A. fumigatus*. Our results suggest, that increased *pksP* expression is responsible for pigment formation in the mycelium. Comparative proteome analysis of the *pkaC1* overexpressing strain and the wild-type strain led to the identification of proteins regulated by the cAMP/PKA signal transduction pathway. We showed, that elevat-

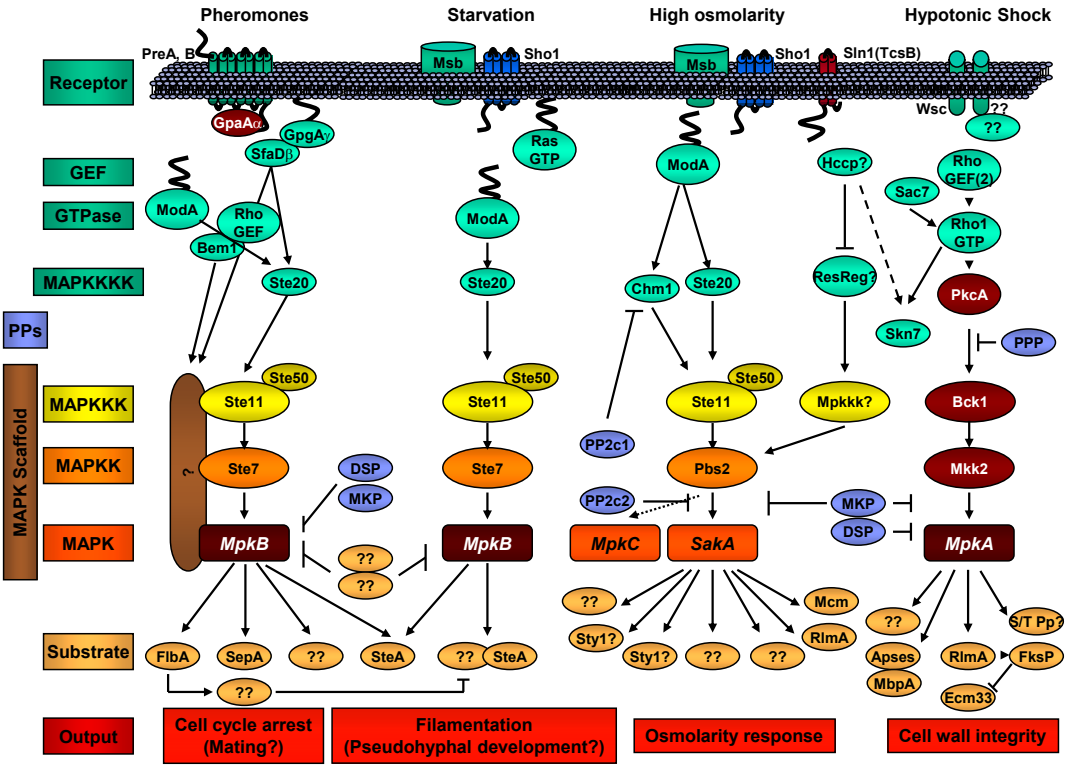


**Figure 3**
MAP Kinase signaling pathways in *Aspergillus fumigatus*.

ed PKA activity resulted in activation of stress-associated proteins and of enzymes involved in protein biosynthesis and glucose catabolism. By contrast, proteins which were involved in nucleotide and amino acid biosynthesis were downregulated as well as enzymes involved in catabolism of carbon sources other than glucose. In additional experiments, deletion of the *A. fumigatus* genes *pdeA* and *pdeB*, encoding phosphodiesterases, similar phenotypes were observed. Therefore, we conclude that growth, sporulation and pigment formation is regulated by cAMP/PKA signaling in *A. fumigatus*.

Mitogen activated protein kinase (MAPK) signaling pathways (Fig. 3) are involved in the regulation of various cellular responses in eukaryotes. In fungal pathogens they are of special interest because of their possible contribution to pathogenicity. Bioinformatic analysis of the genome of *A. fumigatus* revealed the presence of four distinct MAPK-encoding genes (*sakA*, *mpkA*, *mpkB* and *mpkC*). Blast analysis of the MpkA deduced amino acid sequence showed high similarity with MAP kinases acting in the cell wall integrity signaling pathway. We deleted the *mpkA* gene in *A. fumigatus*. *ΔmpkA* mutants showed impairing in growth and development accompanied by restricted elongation of hyphae and elevated branching. *ΔmpkA* mutants were strongly inhibited in growth in presence of cell wall damaging compounds. Western blot analysis revealed that these compounds activate MpkA at post-transcriptional level. To analyse the transcriptional regulation of *mpkA*, an *A. fumigatus* strain carrying the *mpkA* promoter upstream of the *lacZ* gene, was generated. An increase in expression of the gene fusion was detected in response to treatment with different cell wall-damaging compounds. Interestingly, a murine low-dose model for invasive aspergillosis revealed that *ΔmpkA* and wild-type strains presented the same mortality ratio.

### Immune Evasion Strategies of *A. fumigatus*

*A. fumigatus* conidia activate the alternative pathway of the complement system. In order to assess the mechanisms by which *A. fumigatus* evades the activated complement system, in close collaboration with the group of P. Zipfel at the HKI we analysed the binding of host complement regulators to *A. fumigatus*. The binding of factor H and factor H-like protein 1 (FHL-1) from human sera to *A. fumigatus* conidia was shown by adsorption assays and immunostaining. In addition, factor H-related protein 1 (FHR-1) bound to conidia. Adsorption assays with recombinant factor H mutants were used to localise the binding domains. Plasminogen was identified as the fourth host regulatory molecule that binds to *A. fumigatus* conidia. In contrast to conidia, other developmental stages of *A. fumigatus*, like swollen conidia or hyphae, did not bind to factor H, FHR-1, FHL-1, and plasminogen, thus indicating the developmentally regulated expression of *A. fumigatus* surface ligands. Both factor H and plasminogen maintained regulating activity when they were bound to the conidial surface. Bound factor H acted as a cofactor to the factor I-mediated cleavage of C3b. Plasminogen showed proteolytic activity when activated to plasmin by urokinase-type plasminogen activator. These data show that *A. fumigatus* conidia bind to complement regulators, and these bound host regulators may contribute to evasion of a host complement attack.

## 3 Transcription Factors and Signal Transduction / Protein-Protein Interactions

Group Leaders: Peter Hortschansky, Axel A. Brakhage

### Regulation of Fungal Transcription Factor Complexes

Heterotrimeric CCAAT-binding complexes are evolutionary conserved in eukaryotic organisms including fungi, plants and mammals. In the filamentous fungus *Aspergillus nidulans*, an important model organism for fundamental biological questions such as development, gene regulation or the regulation of the production of secondary metabolites, the corresponding complex was designated AnCF (*A. nidulans* CCAAT-binding factor). AnCF consists of the subunits HapB, HapC and HapE. All three subunits are necessary for DNA binding. Although a number of AnCF-regulated genes are known and analyzed so far, e.g. the two penicillin biosynthesis genes *aatA* and *ipnA*, hardly nothing is known about the regulation of the complex itself. Therefore, one of our aims was to investigate which mechanisms might be important for the posttranslational regulation of the complex-assembly and its transcriptional activity and specificity.

### AnCF and HapX: A Novel Mechanism of Gene Regulation by Iron in Eukaryotes

Due to the fact that AnCF can act as an activator or repressor of transcription, it is most likely that AnCF facilitates the association of further transcriptional elements. Recently, a putative fourth AnCF subunit with an unknown function was identified in *A. nidulans* and designated HapX. In collaboration with the group of Hubertus Haas (Biocenter, Innsbruck Medical University), we found that *hapX* expression is repressed by iron via the GATA-factor SreA and that various iron-dependent pathways (e.g., heme biosynthesis) are repressed during iron starvation by the interaction of HapX with the AnCF. These data suggest a model, in which HapX/AnCF interaction is regulated at both transcriptional and post-translational levels. Iron starvation causes expression of *hapX*. Subsequent binding of HapX to AnCF results in transcriptional repression of iron-dependent pathways. During iron-replete conditions, *hapX* is repressed and, therefore, iron-dependent pathways are derepressed. Moreover, HapX/AnCF interaction is inhibited by increased iron concentrations. This post-translational mechanism allows rapid adjustment to iron availability by disruption of the HapX/CBC complex. Mutual transcriptional control of *hapX* and *sreA* coordinates iron acquisition and iron-dependent pathways, thereby serving for both iron supply and prevention of iron toxicity. These data indicate that the AnCF has a general role and that HapX function is confined to iron depleted conditions (Fig. 4).

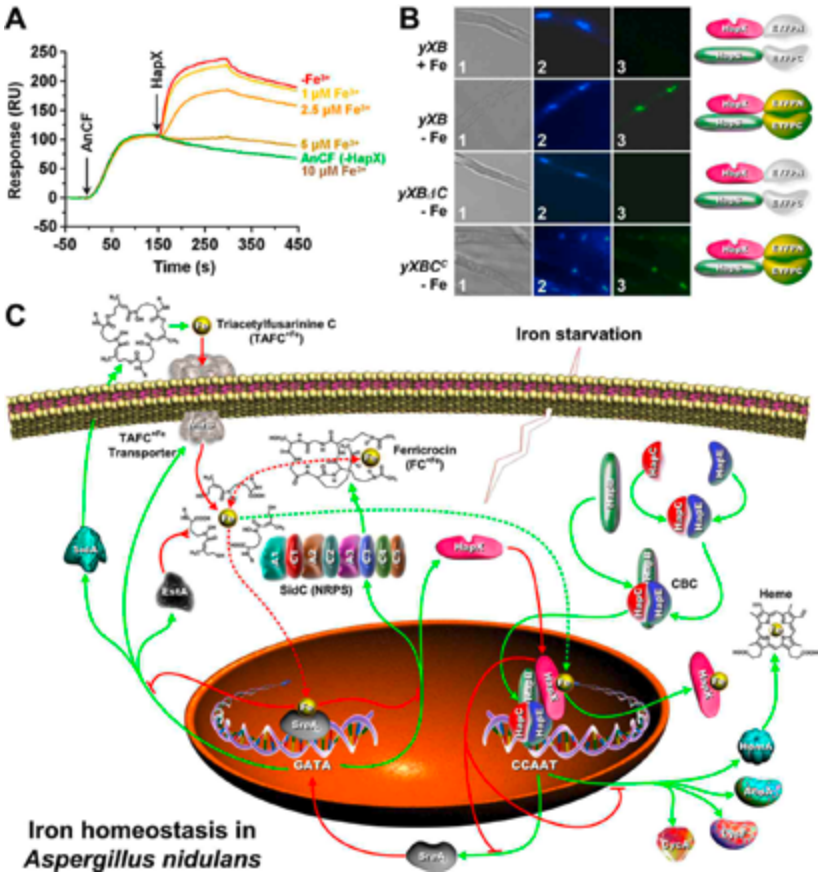


**Figure 4**
Detection of the iron-regulated interaction between HapX and HapB *in vitro* and *in vivo*.
(A) Surface plasmon resonance interaction analysis of 100 nM HapX with DNA-bound AnCF after preincubation without (-$Fe^{3+}$) and with 1, 2.5, 5 and 10 µM $FeCl_3$. (B) Interaction between HapX and HapB was observed using bimolecular fluorescence complementation (BiFC) in *A. nidulans* strains producing HapX and HapB fused with the C-terminal and N-terminal split fragments of enhanced-yellow-fluorescent-protein (EYFP), respectively. Panel 1, light microscopy; panels 2 and 3, fluorescence microscopy of DAPI-stained nuclei and BiFC, respectively. HapX and HapB interact during -Fe but not +Fe conditions in strain yXB. HapX/HapB interaction is abolished by deletion of *hapC* in strain yXBΔC and is reconstituted after complementation of yXBΔC with *hapC* in $yXBC^c$. (C) Proposed model for HapX/AnCF-mediated regulation of iron homeostasis in *A. nidulans*.

### Redox Regulation of the CCAAT-binding Complex is Mediated by the Thioredoxin System

Interestingly, all putative HapC orthologs from other eukaryotes contain three conserved cysteine residues in their core histone fold motif. It is known for the NF-Y complex, the human homologue of AnCF, that oxidative as well as antioxidative conditions may alter the DNA-binding of this transcriptional complex. For a detailed study, all the Hap proteins were overproduced in *E. coli* and purified to homogeneity. Furthermore, by exchanging the three cysteines of HapC against serine in all possible combinations, we were able to create seven additional HapC mutants. Surface plasmon resonance measurements revealed that two of these cysteine residues are indispensable for stable HapC/HapE subcomplex formation and high affinity AnCF DNA-binding.

These *in vitro* data are coincident with HapC-EGFP localisation studies *in vivo*, carried out in an *A. nidulans hapC* deletion strain which in addition, encodes a *hapC-egfp* gene fusion complementing the Δ*hapC* phenotype to wild type. Under reducing conditions, the HapC-EGFP-derived fluorescence was located in the nucleus. By contrast, a HapC-EGFP fusion in which all HapC cysteines were exchanged to serines was located in the cytoplasm, which is in agreement with the impaired stability of the HapC(SSS)/HapE heterodimer. Furthermore, addition of $H_2O_2$ led to cytoplasmic localization of the HapC-EGFP-derived fluorescence which reappeared in the nucleus after 1 hour. Moreover, in a thioredoxin deletion strain, the GFP-fusion of HapC is also mainly located in the cytoplasm.

Therefore we were interested, whether an intracellular oxidoreductase system is involved in the redox regulation of HapC. For this reason, the two genes encoding the major thioredoxin system of *A. nidulans* were cloned and the protein products purified. Thioredoxin systems are comprised of thioredoxin (Trx) and thioredoxin reductase (TrxR) and are widely distributed in

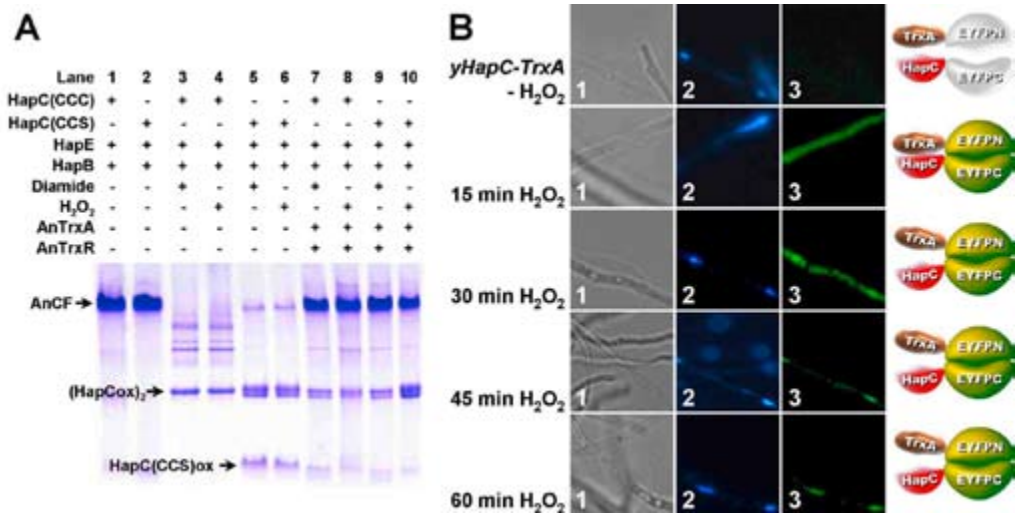


**Figure 5**
Disruption of the AnCF complex by oxidation and recycling by the *A. nidulans* thioredoxin system *in vitro* and *in vivo*. (A) Native PAGE analysis of an oxidation/reduction cycle of AnCF composed of either HapC(CCC) or HapC(CCS) using diamide or $H_2O_2$ for oxidation, respectively. (B) Cellular co-localization and visualization of transient HapC-TrxA interaction under oxidative stress conditions by BiFC in *A. nidulans* producing HapC and TrxA fused with the C-terminal and N-terminal split fragments of EYFP, respectively. Panel 1, light microscopy; panels 2 and 3, fluorescence microscopy of DAPI-stained nuclei and BiFC, respectively.

prokaryotes and eukaryotes. Thioredoxin systems play an important role in maintaining the redox state of the cell and in protecting the cell against oxidative stress by scavenging reactive oxygen species through a variety of mechanisms. The main function of this oxidoreductase system is the reduction of disulfide bridges. In an NADPH-dependent protein disulfide reduction reaction TrxR catalyzes the reduction of oxidized thioredoxin using NADPH as electron donor, and FAD as cofactor. Reduced Trx directly reduces the disulfide in the target protein. When using oxidized HapC as substrate in a coupled NADPH-dependent thioredoxin/thioredoxin reductase assay we were able to reduce the intra- and intermolecular disulfide bridges of HapC. Furthermore, after reduction of the oxidized HapC by the recombinant *A. nidulans* thioredoxin system, HapC was able to participate in the AnCF-assembly by interacting with HapE and HapB. These *in vitro* data imply that the *A. nidulans* thioredoxin system is necessary to keep HapC in its reduced form, and thereby allows HapC to form a functional AnCF together with HapE and HapB. Consistently, a direct interaction of the *A. nidulans* thioredoxin A (AnTrxA) and oxidized HapC was observed in vivo when bimolecular fluorescence complementation (BiFC) experiments were carried out (Fig. 5). Furthermore, northern blot analysis, as well as proteome analysis indicated that a *trxA* deletion strain shows similar expression patterns of AnCF-regulated genes, when compared with *hapE* or *hapC* deletion strains. Taken together, redox regulation via thioredoxin very likely represents a general feature of the CCAAT-binding complex in eukaryotes.

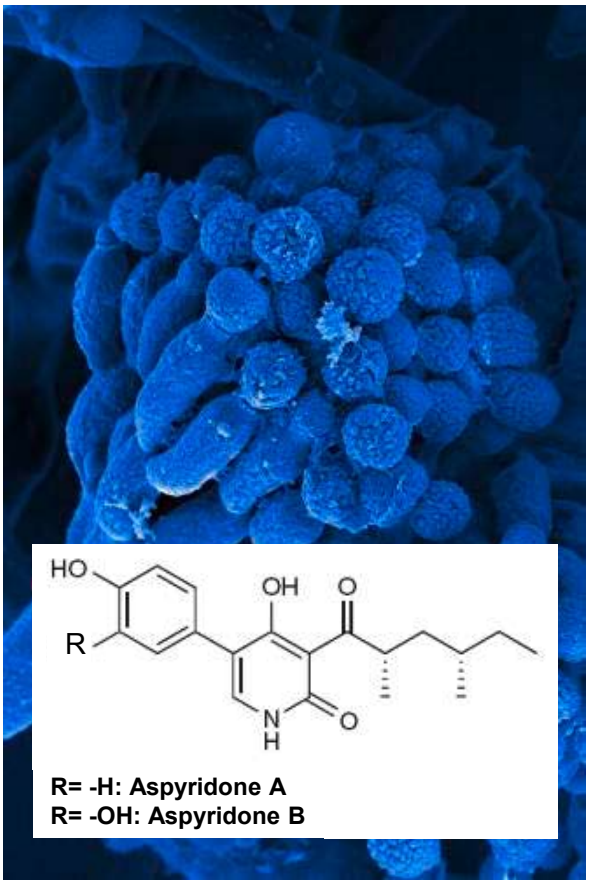


**Figure 6**
Structure of the aspyridones A and B

## 4 Regulation of Fungal Secondary Metabolism Genes

Group Leader: Axel A. Brakhage

### Activation of Fungal Silent Gene Clusters

The formation of many important fungal secondary metabolites, polyketides and nonribosomal peptides, involves multifunctional enzymes: polyketide synthases (PKSs) and nonribosomal peptide synthetases (NRPSs). Mining of the *A. nidulans* genome for gene clusters that could code for the biosynthesis of natural products led to the discovery of a putative hybrid PKS-NRPS gene. Despite vast metabolic data available for *A. nidulans* the role of this gene cluster could not be clarified. The cryptic *A. nidulans* PKS-NRPS gene cluster contains a putative activator gene designated *apdR*. The deduced gene product is related to a putative C6 transcription factor of *A. fumigatus* and to a putative regulator with a GAL4-type $Zn_2Cys_6$ binuclear cluster DNA-binding domain from *A. flavus*. To prove the concept that the homologous overexpression of a regulatory gene can lead to activation of a silent gene cluster the putative activator gene *apdR* was amplified from genomic DNA and cloned into an expression vector carrying the promoter of the alcohol dehydrogenase of *A. nidulans*. This promoter can be induced by the addition of cyclopentanone to the medium and repressed by the use of glucose as the carbon source.

Whereas the transcripts were completely absent in the wild type under both non-inducing and inducing conditions, in the transformant strain under inducing conditions strong mRNA signals were detected for the genes of the cluster including the PKS/NRPS hybrid. This observation further confirmed that under the conditions applied this gene cluster was silent without induction. Furthermore, it also helped to confine the borders of the gene cluster.

HPLC coupled to DAD and MS detectors revealed that, under inducing conditions, the transformants strains produced two main

products as well as two minor compounds. The main metabolites were isolated and their structures were elucidated by 1 and 2D NMR measurements as well as by IR and MS by the group of C. Hertweck. The novel metabolites produced by the transformant strain were named aspyridones A and B due to their pyridone moieties (Fig. 6). In a broad bioactivity screening they exhibited moderate cytotoxic activities. The function of the aspyridones in the natural context and possible triggers for the onset of their biosynthesis remain the subject of ongoing investigations.

However, the results cited here provide the proof of principle for a strategy that may be generally applicable to the activation of silent biosynthesis gene clusters, in particular in eukaroytes. This approach is rendered feasible by the fact that all of the genes encoding the large number of enzymes required for the synthesis of a typical secondary metabolite are clustered and that in some cases, a single regulator controls the expression of all members of a gene cluster to a certain extent. The advantage of this technique is that only a small gene needs to be handled, and that an ectopic integration would be sufficient, bypassing all limitations of homologous recombination. Most conveniently, this strategy would allow for the concerted expression of all pathway genes.

### Identification of New Regulators of the Penicillin Biosynthesis in *A. nidulans*

One focus of our group is the identification and characterisation of signal transduction cascades and regulatory proteins that are involved in the biosynthesis of secondary metabolites of the filamentous fungus *Aspergillus nidulans*. With the protein kinase C PkcA and velvet A (VeA) two regulators of the penicillin biosynthesis could be identified.

It was known that AnBH1, a basic region helix-loop-helix transcription factor characterised by our group, acts as a repressor of the third penicillin biosynthesis gene *aatA*. However, only little information was available on the signal transduction cascade(s) leading to AnBH1. We showed that inhibition of protein kinase C (Pkc) activity in *A. nidulans* led to cytoplasmic localisation of an AnBH1-eGFP protein fusion. Computer analysis of the genome and screening of an *A. nidulans* gene library revealed that the fungus possesses two putative Pkc-encoding genes which we designated *pkcA* and *pkcB*. Only PkcA showed all the characteristic features of fungal Pkcs. Production of *pkcA* antisense RNA in *A. nidulans* led to de-repressed expression of a reporter gene fusion of *aatA*, to reduced penicillin production, and to predominantly cytoplasmic localisation of AnBH1. Therefore, PkcA was shown to be part of the network regulating penicillin biosynthesis. Moreover, it had in impact on growth and conidiation, as is was described for the Pkcs in other fungi.

We showed that the light-dependent regulator velvet A (VeA) acts as a repressor on the penicillin biosynthesis mainly via repression of *acvA*, the first gene of the pathway. VeA was known to be a positive regulator of sexual development in *A. nidulans*. Expression of a regulatable *alcA*p-*veA* gene fusion in an *A. nidulans* strain carrying, in addition, a reporter gene fusion of *acvA* indicated that under *alcA*p-inducing conditions penicillin titres and expression of *acvA* were drastically reduced. The same level of repression was found irrespective whether the *alc*Ap-*veA* gene fusion was expressed in a *veA1* (encoding a truncated protein) or *ΔveA* background, with or without light. However, the expression of the *ipnA* reporter gene fusion was only moderately affected suggesting a less prominent effect of VeA on the second gene of penicillin biosynthesis. Taken together, both studies indicated overlapping regulatory signal cascades in secondary metabolism and developmental processes in *A. nidulans*.

## 5 Systems Biology and Bioinformatics

Group Leader: Reinhard Guthke

### Integrative Bioprocess Data Analysis and Network Inference

The goal in systems biology and bioinformatics is the discovery of structures and the understanding of the dynamics of biological systems. Systems biological research at the HKI is dedicated to the integrated analysis of transcriptome and proteome as well as microbiological

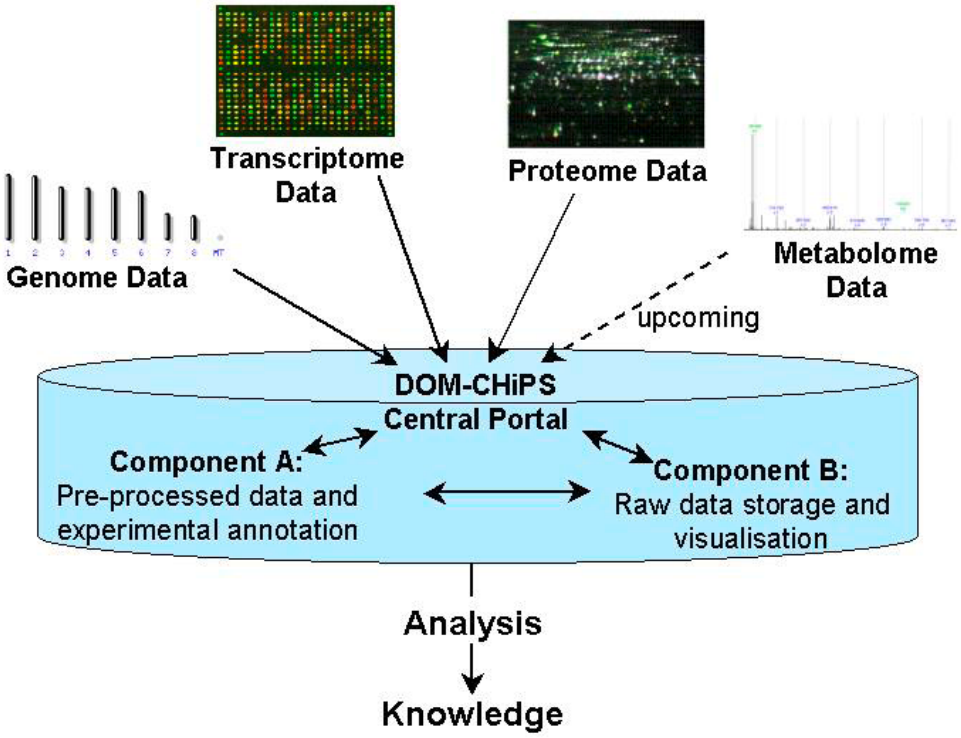


**Figure 7**
Structure of the Data Warehouse DOM-CHiPS. The user communicates via the central portal. Image based raw data, in particular transcriptome and proteome data, are uploaded and pre-processed. Pre-processed data stored together with the experiment annotations and linked with genomic data are provided for visualisation and advanced data analysis.

and clinical data. Data analysis comprises storage and management of heterogeneous experimental data in a data warehouse, data pre-processing, discovery of the main features and patterns and – finally – generation of testable model hypotheses. The hypotheses have been embedded in predictive mathematical and computational models that represent (i) the available expert knowledge, (ii) facts extracted from biological databases and (iii) the hypotheses generated by experimental data analysis. The data analysis pipeline at the HKI is focused on the generation of quantitative dynamic and/or qualitative temporal models that are generated from time series data by dynamic data mining techniques. Some of these techniques have been developed – in collaboration with other groups – by our own group.

Bioinformatics research at the HKI Jena is carried out in the Group 'Systems Biology and Bioinformatics' that is part of the department of Molecular and Applied Microbiology as well as in the Junior Research Group 'Bioinformatics Pattern Recognition'. Both groups are members of the 'Jena Centre for Bioinformatics' (JCB) and the International Leibniz Research School for Microbial and Biomolecular Interactions (ILRS Jena). Furthermore, the 'Systems Biology and Bioinformatics' Group is involved in the DFG Priority Programme 1160 'Colonisation and infection by human-pathogenic fungi', in HKI's *Arthroderma benhamiae* Genome Project as well as in the BMBF-funded German competence network 'Systems of Live – Systems Biology' (HepatoSys).

### Management and Pre-processing of Transcriptome and Proteome Data

A data warehouse for human pathogenic fungi, called DOM-CHiPS (**www.domchips.de**, see Fig. 7), was developed and provided for the members of the DFG Priority Programme 1160. The system was developed to store experimental transcriptomic and proteomic data of different human pathogenic fungi, in particular *Candida albicans* and *Aspergillus fumigatus*, and their

host created by several groups. It allows a unified view via genomic basis data. DOM-CHiPS' component for raw data storage, pre-processing and visualization was developed and is hosted at the HKI (Albrecht et al., 2007). It is based on the database tool PROTECS (Decodon GmbH, Greifswald). It provides tools for uploading and storage of images and corresponding data from microarray or 2D-PAGE experiments and mass spectrometry data that have been pre-processed by image analysis software tools like Array Vision, GenePix, MAS, GCOS, Delta2D, DeCyder or ImageMaster 2D Platinum. Basic genomic data are derived from NCBI GenBank or UniProt. All experimental data are cross-linked via these genomic data. One can find an interesting protein on a gel and then have a look on which other gels in the same or other projects this protein was found and even on which microarrays the respective mRNA probe had been spotted. The GenBank information can be viewed for every gene containing links to the right protein with its UniProt information and again every microarray or gel where this gene or its protein was detected.

For pre-processing and quality control of DNA microarray data Artificial Neural Networks (Multilayer Perceptron) were trained by a resilient backpropagation algorithm comparing the microarray data with qRT-PCR data. Microarray probesets were classified in four categories of reliability for a given experiment. So it is easy to decide whether a probeset can be trusted or not. By comparing how many probesets are classified in certain categories a general quality score of the experiment can be obtained. This software tool was applied, for example, to raw data measured by TIGR's *Aspergillus fumigatus* microarrays version 2 to understand the pathobiology of *A. fumigatus* (calcineurin mutant Δ*calA*). It has been shown that the result of cluster analysis of gene expression data became more stable when the clustering was performed after pre-processing by the Artificial Neural Networks developed. The cross-hybridization on poorly designed microarray probesets were identified as the main component of bias. To reduce the bias caused by cross-hybridizations a 'Boolean Probeset Reassembly' algorithm was developed.

Several algorithms were evaluated for normalization and imputing of proteome data. It was shown that commercial software for proteome data analysis (e.g., DeCyder) is not sufficient to remove all bias from the data. It was found that the combined application of two well-known normalization methods (vsn - variance stabilisation, loess - locally weighted regression) works better than each single normalization method.

### Dynamic Network Reconstruction from Gene Expression Time Series Data

Modeling in Systems Biology comprises a cooperative cycle between model construction and experimental validation. The identification of mathematical and computational models from experimental data by considering the available prior knowledge to generate new hypotheses is the central element in the systems biology cycle. The network inference method NetGenerator implemented and improved by BioControl Jena GmbH was evaluated for several experimental data as well as systematically by artificial (in silico) data (Toepfer et al., 2007; Guthke et al., 2007; Krause et al., 2007). Currently, we design, implement and evaluate methods that integrate prior knowledge into the network inference process. Various mathematical models were generated from gene expression as well as metabolic data monitoring liver cells under defined conditions of externally perturbed cell cultivation. Nonlinear dynamic models with more than 40 differential equations were constructed and fitted to experimental data of 7 and more runs of the human liver cell bioreactor in collaboration with the Charité Berlin (Guthke et al., 2006a; 2006b).

The Lasso algorithm (Least Absolute Shrinkage and Selection Operator) was applied and modified to integrate uncertain biological knowledge supplied by gene regulatory databases into the network inference. Hence, the proposed approach is a data- and knowledge-based network inference algorithm. We found that this new modeling method performs better than other linear model reconstruction techniques. The modified LASSO algorithm was applied to short time-series gene expression data measured by qRT-PCR from 17 pa-

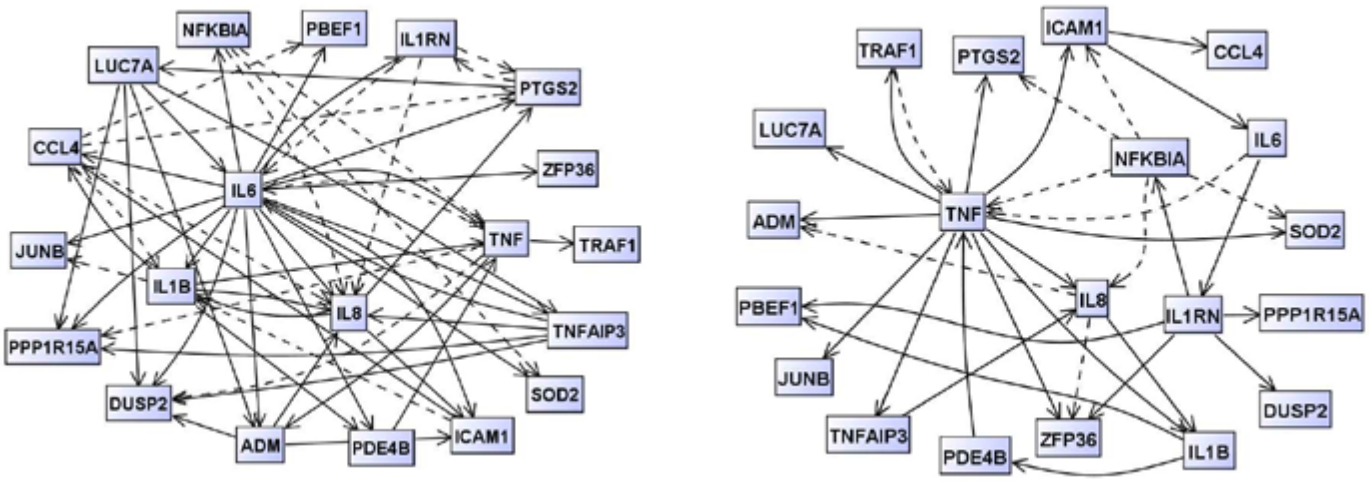


**Figure 8**
Visualization of the inferred dynamic gene regulatory network for the anti-TNF alpha therapy responder group (left) and non-responder group (right) of patients suffering from rheumatoid arthritis. Each gene coding for a cytokine is represented by a node and gene regulatory interactions are shown by directed edges. Solid lines stand for activating effects, while dashed lines stand for inhibitory effects. The network was inferred by the modified LASSO algorithm (Hecker et al., 2006; Koczan et al., 2008).

tients suffering from rheumatoid arthritis. The data monitor the response of patient towards anti-TNF-alpha therapy. It was found that the reconstructed networks differ for patients with different responsiveness to the therapy, where the responsiveness was evaluated by a set of clinical criteria summarized by the so-called Disease Activity Score DAS28. The resulting models (Fig. 8) provide deeper insights into understanding of the therapeutic effect and can lead to the investigation of new drug targets or an individualized therapy (Glocker et al., 2006).

Other model architectures and model inference methods were evaluated for identification of the dynamics and for simulation of the kinetics of gene expression, in particular Dynamic Bayesian Networks, Hidden Markov Models and Formal Concept Analysis. First steps were done to model gene expression profiles describing the stages of infection of liver by *Candida albicans* (Thewes et al., 2007). Quantitative modeling of spatio-temporal data for better understanding of host/pathogen interactions is the topic of a Junior Research Group for Applied Systems Biology prepared in 2006 and 2007 and to be launched in 2008.

Within the German research network on systems biology of hepatocytes (HepatoSys) we investigate the regulation of liver regeneration with focus on the Wnt/beta-catenin signaling and crosstalk to other pathways. We analyzed gene expression profile data from perturbation experiments with murine hepatocytes using knowledge and data based network inference and the Explain Software (BioBase AG). We found strong indication by promoter modeling that transcription factors of the HNF3 (hepatocyte nuclear factor 3) family play a pivotal role in the regulation of genes co-expressed with the beta catenin target gene c-jun.

## Annotation of Fungal Genomes

The *Arthroderma benhamiae* genome project (**http://www.arthroderma.hki-jena.de/**) was launched in 2007 by a consortium of the HKI

(as the leading institution) together with the Leibniz Institute for Age Research – Fritz Lipmann Institute (FLI) and the Friedrich Schiller University Jena. At first, the mitochondrial genome was annotated. Then, we found synteny on large stretches by comparison of the newly sequenced stretches with known genomes of closely related organisms. We performed the gene identification using the gene prediction tool GeneID with support of FLI's Genome Analysis Group. For functional annotation, the new fungal genome browser (FGB) on basis of GenColors (developed at the FLI) has been applied. Particular genes, proteins, intergenic and promoter regions have already been annotated to assist the experimentally working groups of the consortium. We applied the Protein Localization Predictor WolfPsort and SignalP to identify location of signal peptide cleavage sites in amino acid sequences to check potential secretion for all predicted proteins.

Besides the annotation of the *A. benhamiae* genome, the annotation of the genomes of *A. fumigatus* and *A. nidulans* was improved: Transcription factors in *A. fumigatus* were re-annotated. This information was also included in our data warehouse. PKS and NRPS gene clusters and surrounding genes (Shelest et al., 2007) as well other gene clusters of interest (e.g. Penicilin and other secondary metabolite clusters) in *A. fumigatus* and *A. nidulans* were investigated to assist experimentally working groups of the HKI.

## 6 Taxonomy

Group Leaders: Ingrid Groth (until April 2007), Karin Martin

### Evaluation and Standardization of Sampling, Classification of Actinomycetes from Indoor Environments and Determination of the Most Common Actinomycetes in Water Damaged Buildings

Within a joint project headed by the Regierungspräsidium-Stuttgart-Landesgesundheitsamt (LGA) the occurrence of actinomycetes in water damaged buildings was studied. Health problems of people living in microbial contaminated rooms are mainly considered to be caused by moulds. However, despite of the known pathogenic effects of moulds present in indoor environments an unambiguous correlation between their occurrence and the observed complex human health problems is missing. Furthermore it was shown by recent studies that fungal colonization of humid environments is very often accompanied by bacteria and sometimes also by mites. From about 600 samples collected from water damaged indoor environments more than 80 % contained bacteria, among them a great number of isolates belonging to different genera of the order *Actinomycetales*. The aim of the project was to provide praxis relevant methods for the isolation and identification of actinomycetes from contaminated indoor environments as a prerequisite for further taxonomic and infection-biological studies.

### Isolation and Taxonomic Characterization of Actinomycetes

For assessing the human health risk of actinomycetes colonizing water damaged buildings optimal conditions for their isolation and cultivation had to be developed. Therefore, at the beginning of the project a workshop comprising lectures together with an experimental part was organized by the HKI group in January 2006. Living cultures of relevant actinomycete reference strains from the HKI culture collection served to demonstrate the morphological diversity of these organisms and the influence of culture conditions on growth and on the expression of morphological properties. Together with experts from Germany and Finland methods for isolation and a rapid preliminary classification of actinomycete isolates based on morphological and selected biochemical characteristics were discussed and defined.

On the basis of the proposed procedures seven different indoor samples were commonly studied by the partners to compare the efficiency of the applied methods. The actinomycete isolates obtained by the partners were classified by the HKI group according to their morphology and the presence of the isomers of the diaminopimelic acid in the cell wall peptidoglycan. Identical isolates were eliminated and one or two representatives of each taxonomic group were sent to the partner in Giessen for deter-

mination of the 16S rRNA gene sequences. The following 16S rRNA gene sequence comparison allowed an identification of the isolates at the genus or species level.

15 samples from different contaminated sources were studied in 2006/2007. It was found that the culturable actinomycetes revealed a high taxonomic diversity comprising irregular rods or cocci and mycelium forming organisms and that the morphological and selected chemotaxonomic characteristics (diagnostic diaminoacid of the cell wall peptidoglycan, composition of menaquinones and the fatty acid profiles) were in a good agreement with the data of the 16S rRNA gene sequence analysis. The proposed combination of morphological and chemotaxonomic markers contributed in many cases to a preliminary genus affiliation of the isolates while morphological properties alone were only useful to determine the distribution of certain actinomycetes in the relevant sample. Furthermore, it was found that the spectrum of actinomycete genera was strongly influenced by the composition of the samples. For example, isolates resulting from four different samples (plaster, insulating material) were affiliated to 24 genera based on 16S rRNAgene sequence data. These genera represent 16 families of the order *Actinomycetales*. Interestingly only members of three genera belonging to three families were found to be present in all of the four tested samples. A final consideration about the most common actinomycetes in water damaged buildings is still in progress.

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Schwerpunktprogramm 1152: Evolution metabolischer Diversität
Evolution und Funktion von cis-/trans-Elementen pilzlicher Sekundärmetabolismus-Gene am Beispiel der Penicillinbiosynthese
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Schwerpunktprogramm 1160: Kolonisation und Infektion durch humanpathogene Pilze
Teilprojekt: Identification of virulence determinants of the human-pathogenic fungus *Aspergillus fumigatus* by proteome analysis
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Teilprojekt: Holistic approach to genomics of human-pathogenic fungi: Data warehouse for integration of data on transcriptome, proteome and metabolome of *Candida albicans* and *Aspergillus fumigatus*
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Teilprojekt: Criss-Cross talk between filamentous fungi and streptomycetes
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Biochemische Charakterisierung der Glycosyltransferase WbpL von *Pseudomonas aeruginosa* als potentielles Target für neue Antiinfektiva
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BioChancePLUS-2 Verbundprojekt: Individualisierte Medizin: Tool zur Therapieentscheidung - Apherese / Immunadsorptions-Chip
Teilprojekt: Bioinformatische Analysen der RA-Patienten: Identifizierung von Genen und Genprodukten für das Ansprechverhalten zur Apherese-Immunadsorptionstherapie
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Teilprojekt: Quantitative Charakterisierung der Dynamik von Leberzell-Populationen im 3D-Leberzell-Bioreaktor
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Dynamische Modellierung des Wnt/beta-Catenin Signalweges während der Leberregeneration
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European Union
6. Forschungsrahmenprogramm der EU
Specific Targeted Project: Integrating and strengthening the European Research Area
MANASP-Development of novel management strategies for invasive aspergillosis
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Marie Curie Research Training Networks
SIGNALPATH -MAP kinase cascades controlling virulence in fungi: from signals to pathogenicity response
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**Herrmann M**, **Sproete P**, **Brakhage AA** (2006) Protein kinase C (PkcA) of *Aspergillus nidulans* is involved in the penicillin production. *Appl Environ Microbiol* 72, 2957-2970.

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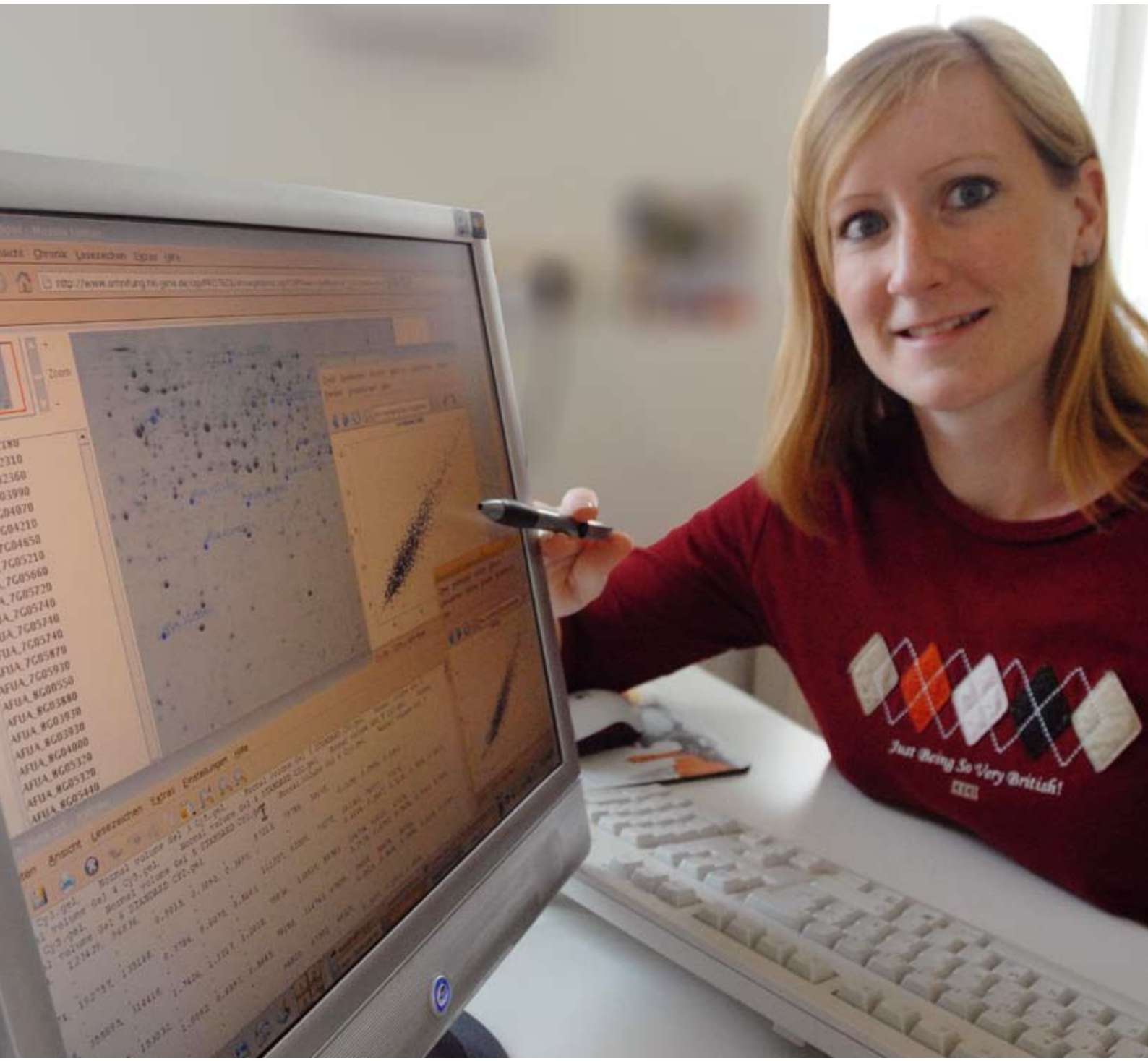


# Junior Research Group
# Bioinformatics – Pattern Recognition

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The junior research group *Bioinformatics – Pattern Recognition* developed and evaluated advanced algorithms by using new computational and statistical techniques to solve formal and practical problems arising from the need for the analysis of complex high-dimensional data. The strongest motivation for our work was the challenge of being able to adequately interpret large-scale genomic expression data from microarrays. A major effort was directed to the increase of the accuracy and trustworthiness of machine learning methods used for data-driven hypotheses generation. This was considered to be a required contribution to facilitate in data-intensive biosciences an effective and efficient hypothesis-driven inference, thus improving models or theories and increasing biological knowledge. Our group represented a project of the Jena Center for Bioinformatics (JCB), a part of the Education and Technology Initiative Bioinformatics of the German Federal Ministry for Education and Research.

In functional genomics and associated fields the determination and test of just a single hypothesis is often time-consuming and costly, in particular, when applying genome-wide expression profiling. If the empirical data are complex, it is largely the computer that must make a decision which hypothesis is most of all worth being tested next. That means, one of the previous domains of human intelligence has to be left to the operation of "artificial intelligence" (AI). This may be critical for two rea-

## INTRODUCTION | EINLEITUNG

**Head:**
**Dr. Ulrich Möller**

Die Nachwuchsgruppe *Bioinformatik – Mustererkennung* entwickelte und evaluierte fortgeschrittene Algorithmen durch Anwendung von rechenbetonten und statistischen Techniken, um formale und praktische Probleme zu lösen, die sich aus dem Bedarf ergeben, komplexe, hochdimensionale Daten auszuwerten. Die stärkste Motivation für unsere Arbeit war die Herausforderung, genomweite Expressionsdaten aus Microarrays adäquat interpretieren zu können. Eines unser Hauptanliegen bestand in der Erhöhung der Genauigkeit und Vertrauenswürdigkeit maschineller Lernmethoden für die datengetriebene Hypothesengenerierung. Dies wurde als notwendiger Beitrag angesehen, um in den daten-intensiven Biowissenschaften eine effektive und effiziente hypothesengeleitete Schlussfolgerung zu fördern und somit Modelle oder Theorien zu verbessern und das biologische Wissen zu erweitern. Unsere Gruppe repräsentierte ein Projekt des Jenaer Centrums für Bioinformatik (JCB) als Teil der Ausbildungs- und Technologie-Initiative Bioinformatik des Bundesministeriums für Bildung und Forschung.

In der funktionellen Genomanalyse und assoziierten Forschungsfeldern sind oft bereits die Festlegung und der Test einer einzigen Hypothese zeitaufwändig und teuer, speziell bei der Anwendung eines genomweiten „Expression Profiling". Sind die empirischen Daten komplex, muss weitgehend der Computer entscheiden, welche Hypothese es am meisten wert ist als nächste getestet zu werden. D.h., eine bisherige Domäne menschlicher Intelligenz muss einer „künstlichen Intelligenz" (KI) überlassen werden. Dies kann aus zweierlei Gründen kritisch

sons. First, hypothesis generation from data is itself an uncertain (inductive) step (inference from an example to the general principle). Second, the success even of the currently best inductive AI methods in a particular application can hardly be predicted; these methods show a data-depending performance and are themselves still an object of research. Therefore, the repertoire of these methods, their performance and the assessment of their results (validation) have to be improved. Our group developed algorithms for the statistical assessment of regularities (patterns) found in sample data (section 1). We created methods to replace heuristic decision-making by optimized automatic parameter-setting for the most widely used fuzzy clustering tool (section 2). Furthermore, we combined these and other methods in multi-step procedures and demonstrated their potential for the discovery of prognostic disease subtypes in gene expression data (section 3). Finally, the group was involved in several interdisciplinary research activities (section 4).

sein. Erstens ist die Hypothesengenerierung aus Daten an sich ein unsicherer (induktiver) Schritt (Schlussfolgerung vom Beispiel zum allgemeinen Prinzip). Zweitens ist der Erfolg selbst der besten induktiven KI-Methoden im Einzelfall kaum vorhersagbar; das Verhalten dieser Methoden ist datenabhängig und die Methoden sind selbst noch Gegenstand der Forschung. Deswegen müssen das Repertoire dieser Methoden, ihre Leistungsfähigkeit und die Evaluierung ihrer Ergebnisse verbessert werden. Unsere Gruppe entwickelte Algorithmen für die statistische Prüfung von Regelmäßigkeiten (Mustern), die in Daten (Stichproben) gefunden wurden (Abschnitt 1). Wir entwarfen Methoden, um bei dem am häufigsten verwendeten Werkzeug zum unscharfen (engl. *fuzzy*) Clustering heuristische Entscheidungen durch eine optimierte automatische Parametrisierung zu ersetzen (Abschnitt 2). Weiter kombinierten wir diese und andere Methoden in Mehrschrittverfahren und demonstrierten deren Potenzial für die Aufdeckung prognostisch relevanter Erkrankungstypen anhand von Genexpressionsdaten (Abschnitt 3). Schließlich war die Gruppe in eine Reihe interdisziplinärer Forschungsaktivitäten involviert (Abschnitt 4).

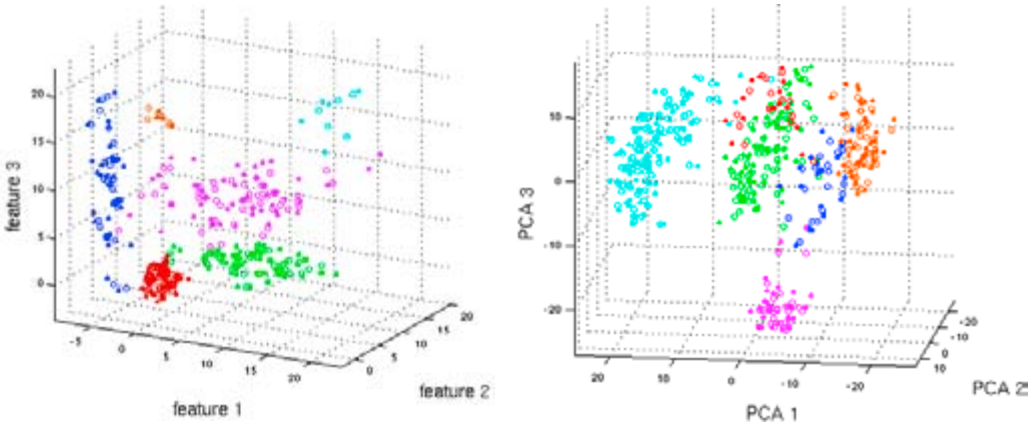



**Figure 1**
Demonstration of nearest neighbor resampling for two data sets representing objects of six (color-coded) classes, respectively. In comparison to the original sample (fat dots), the resample (circles) looks like another random sample. Within a class, the sample and the resample show random differences, while the inter-class difference (the information of interest) were preserved by the resampling method, even though the classes have different characteristics which are unknown to the resampling method. *Left:* stochastic model containing six populations with different distributions. *Right:* gene expression data obtained from six clinically annotated classes of leukemia patients. For the presentation, the expression data of 985 genes were reduced to the data of the three largest principal components (PCA).

## 1 Data Resampling – Methods for the Statistical Validation of Machine Learning Results Obtained from Gene Expression Profiling

Biological data are typically used to generate hypotheses about the underlying population(s). In this sense, a data set obtained from a number of individuals is a random sample; facts derived from these data can be random as well. However, the information of interest is the set of non-random properties that characterize the population. The determination of these properties is the task of a statistical validation. This is a relatively straightforward process when investigating a feature that yields for each sample member a single value so that these values can be subject to a statistical (hypothesis) test. A bigger problem occurs when the entire sample yields just a single (multi-dimensional) feature value. This applies if the feature is a subdivision of sample elements into classes (partition) which can be interpreted as a hypothesis of the existence of general categories (e.g., taxa, subtypes of a disease, or sets of co-regulated genes or gene products of a species). To investigate the significance of such a result, it was recommended to analyze a collection of samples (i.e., a sample consisting of samples). However, due to high costs only one sample is often available, especially in expression profiling with microarrays. Therefore, a simulation, called resampling, is performed: drawing random samples from a data pool which has been built based on the information of a single, true (biological) sample. A key point here is the minimization of a possible systematic error introduced by the simulation procedure into the subsequent results.

The junior research group developed a method that can be applied to test the quality of clustering results depending on a given resampling technique. This test is algorithmically expensive, because it has to be ensured that the resampling performance score obtained is robust against the choice of the clustering algorithm(s)

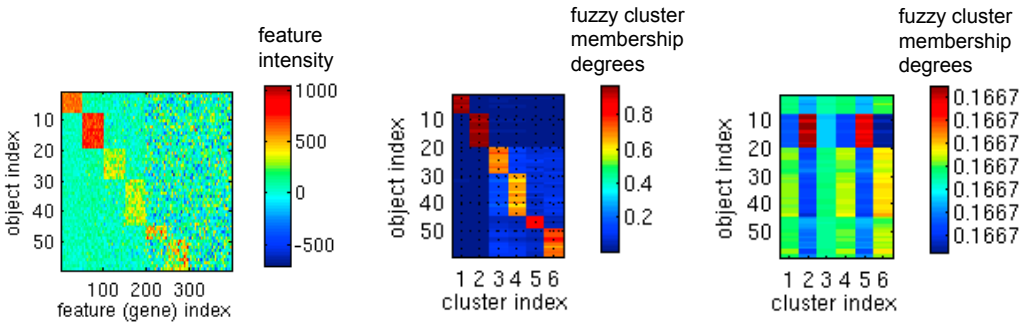



**Figure 2**
Results of two methods for the data-dependent adjustment of the fuzzy C-means clustering algorithm. *Left:* data set consisting of the values of 400 features obtained from 59 objects that belong to six classes (recognizable by the blocking structure). *Middle:* Fuzzy cluster membership degrees obtained by the new mathematical method. The membership degrees of each object correspond with the degree of similarity of the object to the objects of the respective classes. *Right:* Fuzzy cluster membership degrees obtained by the most common heuristic method. All membership values are very similar. The data structure cannot be deduced from the estimated membership degrees.

used for the test. We used our method for the benchmarking of three resampling schemes recently introduced into bioinformatics, in particular, to statistically evaluate clustering results of sparse samples. We were able to give a clear recommendation for the choice of a resampling scheme, where all original data points are randomly 'perturbed' – in contrast to two popular techniques called bootstrapping and subsampling, where a randomly selected part of the original data is omitted in each resample. As another conclusion from our study, the group developed a new resampling technique which is called nearest neighbor resampling – NNR (Fig. 1). When applied to results of gene expression profiling, NNR guided the selection of hypotheses for the existence of breast cancer subtypes of potential diagnostic and prognostic relevance (section 3).

## 2 Automatic Tools for the Safe and Robust Determination of Individual Degrees of Membership of Genomic Profiles in Profile Clusters

While the most commonly used clustering methods *fully* assign a data object to one group, fuzzy clustering (FC) allows the *partial* membership of an object in *more than one* group, where the partial membership degrees are estimated from the data. This application of fuzzy logic permits a more realistic modelling of biological and medical data and their underlying subject matter. The choice of hard (as opposed to fuzzy) assignments is arguably more owing to conceptual simplicity rather than intrinsic merits. In medical applications hard assignments leave a lot of information unused thereby increasing the risk of unnecessary faults. In genomic research FC can help, for example, to model the involvement of a gene in several functions. The most frequently used FC method is the fuzzy C-means (FCM) algorithm, a robust "work horse"

in many scientific fields. Nevertheless, the FCM has not yet been established as a routine tool in bioinformatics. This may be partly due to a difficulty of adjusting the FCM properly for the analysis of any particular data set. Since the introduction of the FCM more than 25 years ago, the algorithm's so-called fuzziness parameter has been mostly adjusted according to heuristic rules. However, in 2003 these rules have been shown to be inappropriate for the analysis of gene expression data. The junior research group developed a new automatic method that solves this data-dependent problem. By using, for example, simulated data and gene expression data of cancer, the new method was demonstrated to work safe and robust, while five other computational methods, most of them published during the last five years, did not solve the problem satisfactorily (Fig. 2).

In a diploma thesis Cathleen Marx investigated the practical relevance of the new method using microarray data obtained from the human-pathogenic fungus *Aspergillus fumigatus* and from patients with arthritic diseases. She showed that biologically or medically more plausible results can be generated when the FCM is adjusted based on the new mathematical rather than the common heuristic rules. The new method enabled the use of the FCM for the generation of hypotheses about previously unknown prognostic subtypes of breast cancer (section 3).

## 3 Discovery of Prognostic Disease Subtypes Based on New Methods for the Analysis of Gene Expression Data and Survival Data

One goal associated with gene expression profiling is the discovery of diagnostic and/or prognostic subgroups of diseases. This work has been mainly focused on cancer due to the availability of data. However, the classification also of severe fungal infections is desired and is on the agenda of the HKI research. Based on the assumption that patterns of, e.g., leukemia subtypes, are present in expression data, inconsistent results in the literature suggested that the detection of these patterns may require improved tools for data analysis. To this end, multi-step procedures, consisting of several types of machine learning methods, were developed in our group. These methods were applied to publicly available microarray data sets and survival data obtained from samples of tumor patients.

For each of three categories, breast cancer, lung cancer and ovarian cancer, two or more groups of patients were found who robustly clustered together based on gene expression data. A number of these groups differed significantly in their prognostic profiles (Fig. 3). The prognostic relevance of these profiles was higher as compared to the profiles previously published as the results of more conventional data analysis methods. Moreover, the investigations revealed potential marker genes that were partly unknown and partly known to be oncologically relevant (diploma thesis in bioinformatics of Berit Ulbrich, gene annotation in collaboration with C. Clement, Clinical Research Center, Friedrich Schiller University Jena).

A related multi-step procedure was applied to expression data of genes with high scores in a Cox proportional hazard model (i.e., gene expression correlated significantly with survival time). The data came from four published studies including more than 500 (estrogen receptor positive) breast cancer patients, where alternative microarray platforms (Affymetrix and Agilent) have been used in two pairs of studies, respectively. We found gene expression patterns associated with significant prognostic profiles. The expression patterns were largely consistent across the four different studies and the two different microarray platforms (diploma thesis in bioinformatics of Wiebke Iffert).

Our multi-step procedures (including the methods in section 1 and 2) are prototypical examples of data processing lines that may improve inductive inference from a combination of molecular biological data and survival data.

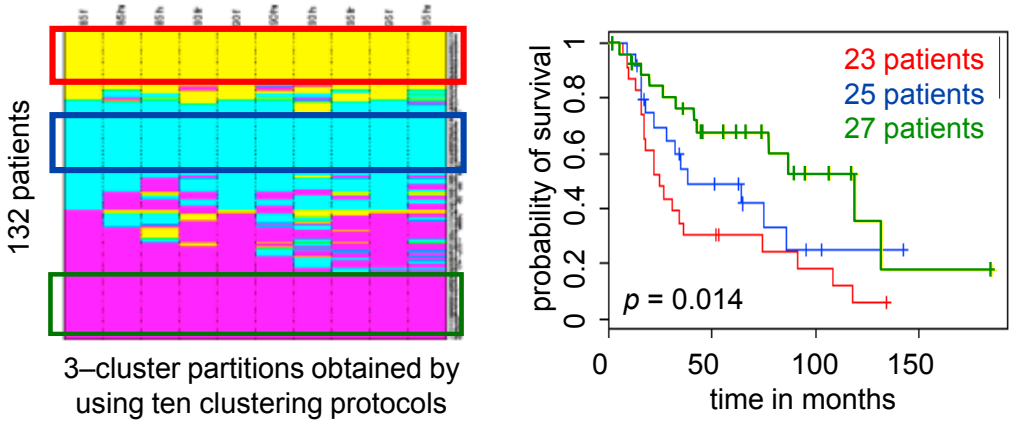


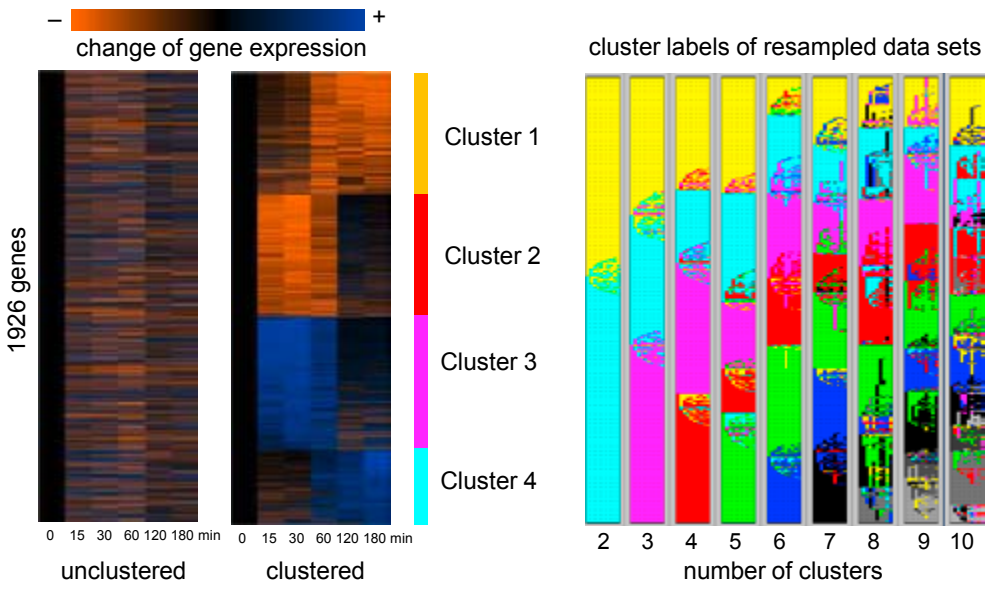

**Figure 3**
*Left:* Color-coded cluster memberships of aligned partitions obtained for gene expression profiles from 132 ovarian cancer patients. The survival time data associated with each of the ten 3-cluster partitions led to a significant Kaplan-Meier-Analysis (KMA). Three patient groups consisting of 23, 25 and 27 patients were assigned to the same cluster, respectively, in each of the ten partitions (see superimposed colored rectangles). *Right:* KMA result for the conserved patient groups that represent distinct prognoses: patients with a gene expression profile similar to the expression pattern of the red, blue and green cluster have an estimated survival probability after five years equal to 30%, 50% and 70%, respectively.

## 4 Applications – Interdisciplinary Work

A current research topic was the development of a method that can be used to predict transcription factor (TF) target interactions for the investigation of those interactions in model organisms and humans. Data from chromatin immunoprecipitation (chIP-chip) experiments are not sufficient for this purpose, because chromatin binding does not necessarily imply regulation, and binding may be difficult to detect if it is condition or cofactor dependent. However, TFs can be reliably assigned to target genes if many lines of direct and indirect evidence are integrated into a single probabilistic model. Our group contributed to such a model developed for yeast, where evidence of gene coexpression was obtained based on a fuzzy clustering analysis of microarray data covering a broad range of cellular functions (collaboration with A. Beyer and T. Wilhelm, Theoretical Systems Biology group, Leibniz Institute for Age Research - Fritz Lipmann Institute, Jena). Pooling the high-confidence interactions revealed a large network containing 363 significant sets of factors (TF modules) that cooperate to regulate common target genes. In addition, the method predicted 980 novel binding interactions with high confidence that are likely to occur in so-far untested conditions.

The development of a mathematical method called Robust Computational Reconstitution was continued and completed. This method was used for a comparative analysis of gene expression profiles in whole tissues and isolated cell fractions (macrophages, fibroblasts and non-adherent cells) obtained from patients with rheumatoid arthritis and osteoarthritis (collaboration with R.W. Kinne, Experimental Rheumatology Unit, Friedrich Schiller University of Jena and the group of H.-J. Thiesen, Institute of Immunology, University of Rostock). The method can be used to investigate cell type-specific gene expression under different

**Figure 4**
*Left:* display of the temporal changes of gene expression data in *Aspergillus fumigatus* after a temperature shift. *Right:* Aligned cluster assignments for ten data sets generated by nearest neighbor resampling. According to a computational assessment of high cluster stability, the consensus partitions with four clusters was the finest structure with robust results.




conditions. This is a central aim of biomedical research, because biological tissues consist of various cell types that differentially contribute to physiological and pathophysiological processes.

To understand the molecular mechanisms in human-pathogenic fungi the HKI also developed approaches for systems biology. Large-scale gene expression time profiles of *Aspergillus fumigatus*, recorded during a temperature shift (stress response), were analyzed to fit gene regulatory network models by reverse engineering. However, the common limitation of the expression profiling to a few time points restricts the number of interactions that can be reliably estimated from the data. To enable the modeling of the most relevant interactions, our group applied methods for the extraction of characteristic gene expression signatures (Fig. 4). This included nearest neighbor resampling (section 1), optimized fuzzy clustering (section 2) and several cluster validation methods (collaboration with R. Guthke, HKI Systems Biology Group). The models obtained when using different sets of expression signatures provided specific hypotheses about the gene regulation in *A. fumigatus* which are worth being tested experimentally.

The junior research group contributed to several further research activities in different fields. Processes of inflammation, atherosclerosis and aging were investigated based on genom-wide expression profiling (collaboration with A. Habenicht, Institute of Vascular Medicine of the FSU Jena).
Enzyme kinetics were investigated and modeled in order to determine the recombinant expression of protease from the Tobacco Etch Virus (HKI-intern collaboration with M. Kraft and U. Horn, Bio Pilot Plant and P. Zipfel, Dept. of Infection Biology).

Methods developed in our group for the analysis of genomic expression data were also suc-

cessfully applied to improve the accuracy of inductive inference from other types of complex biological data. This was confirmed for the problem of class discovery in a set of profiles generated by the application of Raman spectroscopy to bacteria of different species, genera and strains (collaboration with P. Rösch, J. Popp, Institute for Physical Chemistry, Friedrich Schiller University of Jena).

For the determination of target genes of the classical and an alternative NF-κβ signaling pathway we analyzed gene expression data describing the signal transduction after stimulation of the lymphotoxin-β receptor in a mouse model (collaboration with A. Lovas, F. Weih, Leibniz Institute for Age Research – Fritz Lipmann Institute, Jena).

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## Selected publications
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## Collaborations

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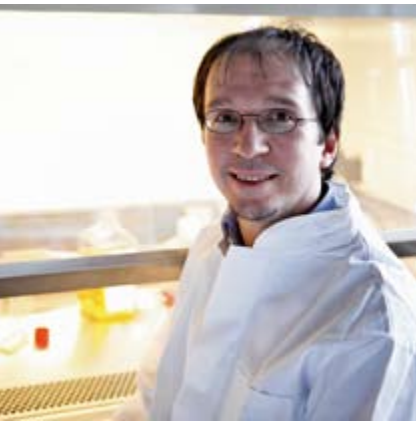
# Junior Research Group Cellular Immunobiology

# Junior Research Group
# Cellular Immunobiology



The various cellular and soluble components of the innate immune system constitute the first line of defense against microorganisms. This system is responsible for differentiating self from non-self, to eliminate the recognized targets, and also to help the adaptive immune response. The Junior Research Group Cellular Immunobiology addresses the role of soluble immune mediators as well as innate immune cells, such as neutrophil granulocytes and macrophages, in innate host defense. In addition, we are interested in malfunctions of defense mechanisms and are investigating the role of anti-complement autoantibodies in human diseases.

*Candida albicans* is a commensal human-pathogenic fungus and causes severe infections in immunocompromised individuals. Thus, fungi are seen continuously by the immune system, and inflammation is controlled to prevent host tissue damage. Therefore, we are studying the physiological functions of innate immune cells during recognition and cellular responses to the pathogen.

## INTRODUCTION | EINLEITUNG

**Head:**
**Dr. Mihály Józsi**

Molekulare und zelluläre Bestandteile des angeborenen menschlichen Immunsystems sind für die ersten Abwehrreaktionen gegen Mikroorganismen verantwortlich. Die angeborene Immunität ist wichtig für die Unterscheidung zwischen körpereigenen und fremden Molekülen und Partikeln, für die Eliminierung der erkannten Zielstrukturen, aber auch für eine effektivere adaptive Immunantwort. Die Nachwuchsgruppe Zelluläre Immunbiologie befasst sich mit der Rolle von molekularen und zellulären Bestandteilen des angeborenen Immunsystems, wie etwa Komplement, Makrophagen und neutrophilen Granulozyten. Zusätzlich haben wir Interesse an funktionalen Defekten in Abwehrprozessen und untersuchen die Rolle von anti-Komplement Autoantikörpern in humanen Erkrankungen.

*Candida albicans* ist ein kommensaler humanpathogener Pilz, der bei immun-supprimierten Menschen schwere Infektionen verursacht. Da kommensale Pilze ständig Kontakt mit unserem Immunsystem haben, muss die inflammatorische Wirtsreaktion kontrolliert werden, um Gewebeschädigungen zu verhindern. Wir analysieren die physiologischen Funktionen von Zellen der angeborenen Immunität während der Erkennung von Pathogenen.

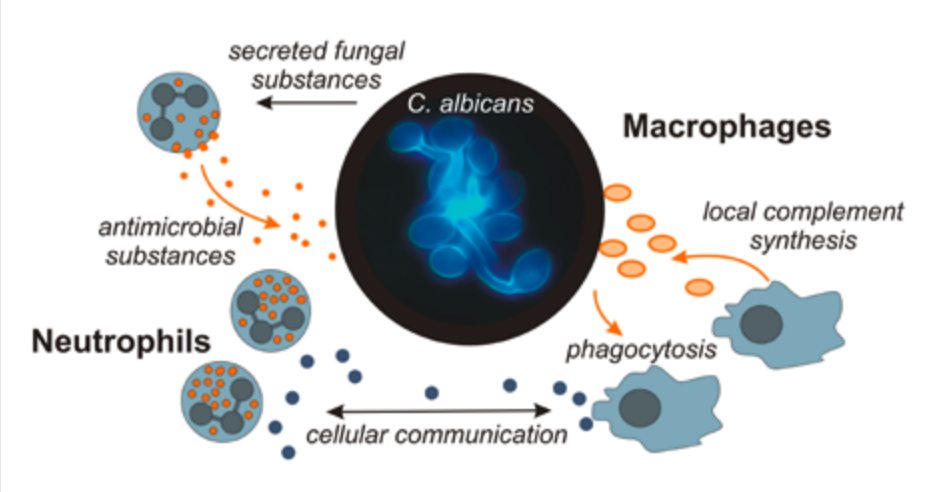


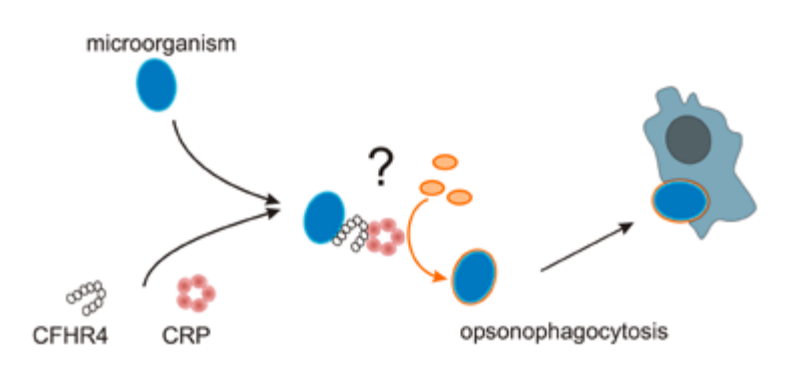

**Figure 1**
Interaction of *Candida albicans* with cells of the innate immune system. Microbes are recognized by immune cells *via* microbial molecules (pattern recognition receptors) or through host molecules bound on the surface of microbes (opsonic receptors). We are studying the roles of human neutrophil granulocytes and macrophages in antifungal defense, and complement in the process of local opsonization.

## 1 Interaction of Innate Immune Cells with *Candida albicans*

Both macrophages and neutrophils are innate immune cells, which play an important role in antifungal defense (Fig. 1). Macrophages are phagocytic cells, which reside in tissues throughout the body. Macrophages function as sentinels of invading microbes and, by "raising alarm" through molecular communication, they attract immune cells, such as neutrophils and lymphocytes, to the site of pathogen entry and inflammation. Furthermore, as professional phagocytes and antigen presenting cells, they shape the adaptive immune response by activating antigen-specific T cells. Neutrophil granulocytes make up 50–70 % of leukocytes in human blood. Neutrophils can actively migrate into tissues to inflammation sites, and effectively phagocytose and kill microbes. Immune cells recognize microbes by pattern recognition receptors that bind to specific molecules on microbial surfaces. These receptors mediate phagocytosis and/or initiate various killing mechanisms or the secretion of cytokines. Neutrophils are rich in granules, which contain preformed mediators and antimicrobial substances. Activation of the cells results in the release of these effector molecules.

Macrophages are also capable of secretion of soluble recognition molecules, such as complement proteins. Locally produced complement C3 may deposit on adjacent microbes (opsonization), and thus facilitate the recognition and uptake of such marked cells via complement receptors (Fig. 1). In this project, we are studying the capability of *C. albicans* to induce complement synthesis in human monocytes and macrophages. To this end, monocytes isolated from human blood are cultured in serum-free medium and are co-incubated with *C. albicans* yeasts and filaments. We aim at identifying the macrophage receptors and signaling pathways, as well as the pathogen molecules responsible for induction of C3 pro-

**Figure 2**
Potential role of the factor H-related protein CFHR4 in opsonization of microbes and dead host cells by interacting with C-reactive protein (CRP).



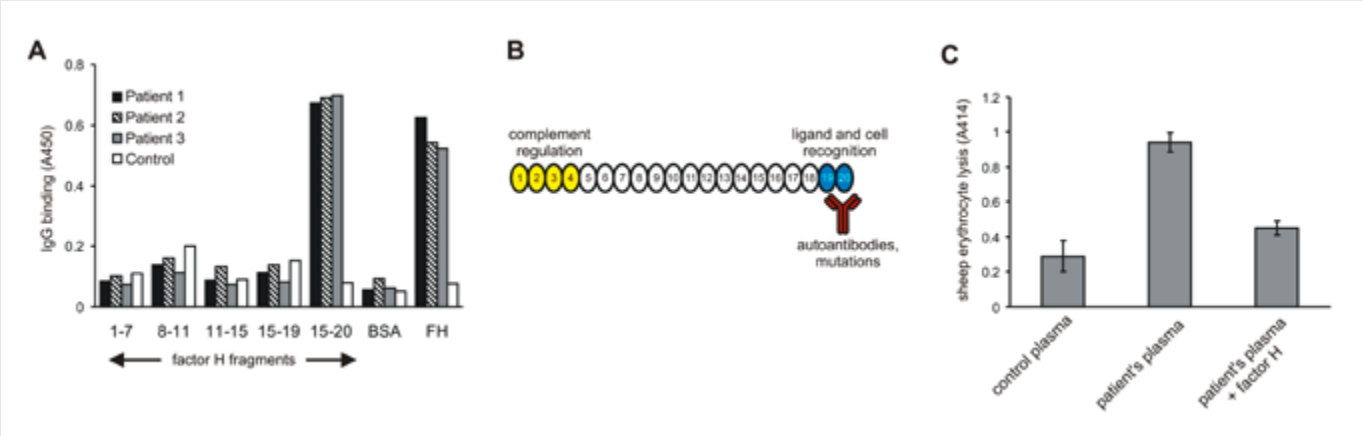




**Figure 3**
Role of factor H autoantibodies in atypical hemolytic uremic syndrome (aHUS). A. Autoantibodies bind to the most C-terminal domain 20 of factor H, as determined using recombinant fragments covering the whole factor H molecule. B. Factor H has two major, well separated functional regions. The four N-terminal and the two C-terminal domains are responsible for complement regulatory activity and for cell surface and ligand recognition, respectively. The C-terminus represents a hot spot for aHUS-associated mutations and also the binding site for autoantibodies. C. Plasma of aHUS patients cause enhanced hemolysis of sheep erythrocytes. The erythrocytes are rescued by addition of purified factor H.

duction. The relevance of complement as local immunomodulator is analyzed in functional assays.

Fungi display on their surface and secrete molecules that function as immunostimulants or immunosuppressants. The immunomodulatory effects of fungal proteins are analyzed in cooperation with the Department of Infection Biology. Pra1p (pH-regulated antigen 1) has been identified in *Candida albicans* as a surface protein, which is highly expressed and secreted by *C. albicans* hyphae. Thus, this protein may play a role in host cellular responses to different morphological forms of the fungus. The project aims at the analysis of the binding and functional effects of Pra1p on human neutrophils and macrophages. Pra1p is shown to bind to neutrophil granulocytes through a specific cellular receptor. We are studying the functional effects of recombinant Pra1p in adhesion and migration assays, to determine whether it works as an attractant molecule for innate immune cells. In addition, the release of antimicrobial substances and cytokines upon exposure to Pra1p is addressed.

## 2 Interaction of Complement Factor H-related Protein 4 and C-reactive Protein During Inflammation and Opsonization

Opsonization is a process during which foreign or modified self particles (such as apoptotic and necrotic cells) are marked with host molecules in order to facilitate recognition and uptake by phagocytes. Major opsonins of the innate immune system include proteolytic fragments of various complement components and proteins of the pentraxin family. The pentraxin C-reactive protein (CRP) is an acute phase protein, which is highly upregulated during inflammation and sepsis, and can reach serum levels of 500 µg/ml. CRP can bind directly to microbes and to apoptotic and necrotic cells. CRP also activates the comple-

ment system and thus generates further opsonins. However, to prevent the over-activation of complement, CRP binds complement regulators such as factor H. Native CRP is composed of five identical subunits that build up a pentameric structure. A denatured, monomeric CRP form can be generated *in vitro*, but its physiological role is unclear.

In collaboration with the Department of Infection Biology we are analyzing the interaction of the two CRP forms with factor H and with factor H-related protein 4 (CFHR4), which we have cloned recently. Because CFHR4 shares related domains with factor H, we hypothesize that CFHR4 is also a CRP binding protein, and modulates opsonization via CRP binding. We propose that by binding to CRP, CFHR4 and other factor H family proteins influence opsonization of microbes and dead host cells, and enhance uptake and safe removal of these opsonized particles (Fig. 2). In addition, we have cloned two murine proteins related to mouse factor H, which may also function as modulators of opsonization.

## 3 Autoantibodies in Kidney Diseases

Molecules of the innate immune system appear as autoantigens in several autoimmune diseases. Recently, an autoimmune form of the kidney disease atypical hemolytic uremic syndrome (aHUS) has been described, where autoantibodies against the complement regulator factor H are present. aHUS can be triggered by various conditions (e.g. infection, pregnancy, drugs), and is associated with defective complement regulation.

Complement is an ancient and promptly acting system of innate immunity, which recognizes and eliminates microorganisms, initiates inflammation and also modulates adaptive immune responses. Complement is composed of ca. 35 components which are present in body fluids and on cell membranes. Activation of this protein network is initiated via target recognition and leads to three activation cascades, which merge in a common terminal pathway and can lead to lysis of microbes through pore formation. However, at the same time complement activation is tightly regulated by both fluid-phase and membrane anchored regulators in the host in order to prevent collateral tissue damage.

In aHUS patients, mutations have been identified in genes coding for such regulators, and these mutations affect the efficiency of complement regulation. In contrast to the genetic factors, the aquired factor H autoantibodies have not yet been analyzed in detail. Thus, our aim is to understand the role of factor H autoantibodies in aHUS. In collaboration with the Department of Infection Biology and with clinicians, we are analyzing how autoantibodies affect the physiologic function of factor H and thus lead to disease.

Factor H is a plasma complement regulator, which is built up from 20 globular domains. The four N-terminal domains are responsible for the complement inhibitory activity of factor H. The majority of aHUS-associated factor H mutations, however, affect the C-terminal domains. We and others have shown, using recombinant mutant factor H proteins and monoclonal antibodies that bind to the C-terminus, that the C-terminus of factor H is critical for the recognition and protection of self cells. Through the C-terminal domains, factor H binds to glycosaminoglycans on host cell surfaces and protects the cells from complement mediated damage. By contrast, factor H cannot bind to microbes which lack cell surface glycosaminoglycans and thus microorganisms are attacked by complement. These results led us to hypothesize that factor H autoantibodies block C-terminal recognition functions of factor H.

We have successfully established an ELISA-based test system in order to screen aHUS plasma samples for the presence of factor H autoantibodies, and so far we have identified 19 autoantibody positive patients. For all analyzed autoantibodies the major binding site was localized to the most C-terminal domain of factor H, using recombinant factor H fragments (Fig. 3A and 3B). Plasma samples of aHUS patients were also analyzed in an *in vitro* cell protection assay using sheep erythrocytes as model for host cells. Sheep erythrocytes, similar to human cells, have polyanionic molecules on their surface, which allow

binding of factor H from human plasma. Thus, in normal human plasma sheep erythrocytes are not lysed by complement. Plasma samples of patients, however, caused enhanced hemolysis of sheep erythrocytes, and the hemolytic effect could be reversed by adding purified factor H (Fig. 3C). These results indicate that the autoantibodies in aHUS patients inhibit factor H binding and thus its protective activity on self cells.

By analyzing autoantibodies in other kidney diseases, the question will be addressed whether factor H autoantibodies are specific to aHUS. In addition, we have observed that the development of autoantibodies is genetically predisposed as in the majority of patients the factor H-related genes *CFHR1* and *CFHR3* are deleted. In conclusion, our data suggest a pathogenic role of factor H autoantibodies in aHUS, and that aiming at the reduction of the autoantibody level may be beneficial to patients.

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Excellence Graduate School JSMC –
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Teilprojekt: Crosstalk between innate pathogen sensing molecules
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## Selected publications

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# Junior Research Group
# Microbial Biochemistry and Physiology

# Junior Research Group
# Microbial Biochemistry and Physiology

The number of infections with pathogenic fungi in patients with a suppressed immune system is steadily increasing. This is mainly due to enhanced techniques in solid organ transplantation, the therapy of patients with leukaemia and the increasing number of patients with HIV infections. The suppression of the innate immune response eases the colonisation of epithelia and allows pathogenic fungi to invade tissues (Fig. 1) and to disseminate within the host.

In order to grow, pathogenic fungi have to acquire nutrients, which are required for growth and for entering new tissues. For that purpose fungi have to be able to metabolise the existent nutrients and have to synthesis metabolites *"de novo"*, which are not present at the respective host site. The understanding of the metabolic processes active during infection could be used for the development of new antifungal compounds, which negatively act on fungal growth. At present only a limited number of antifungal compounds is available which can be used for therapy of invasive fungal infections. However, the use of these drugs is often hampered by severe side effects. Therefore, it appears as an important need to define new compounds, which act specifically on fungi but not humans. Unfortunately, details on the existing nutrients, which can be utilised by fungi, are hardly known and, furthermore, the fungal pathways involved in the metabolism of these nutrients are still under investigated.

Our group currently deals with the pathogenesis caused by the filamentous fungi *Aspergillus fumigatus* and *Aspergillus terreus*. These fungi are ubiquitous distributed, have a very high sporulation capacity and spores can eas-

## INTRODUCTION | EINLEITUNG

**Head:**
**Dr. Matthias Brock**

Die Zahl der Infektionen, die durch Pilze hervorgerufen werden, und hauptsächlich immunsuppremierte Patienten betreffen, ist stetig steigend. Dies liegt an den verbesserten Techniken Organtransplantationen durchzuführen, an der Therapie von an Blutkrebs erkrankten Personen und der steigenden Anzahl HIV-infizierter Personen. Die Unterdrückung des angeborenen Immunsystems erleichtert pathogenen Pilzen die Besiedelung von Epithelien und ermöglicht das Eindringen und invasive Wachstum in Wirtsorganen (Abb. 1).

Pathogene Pilze müssen im Wirt Nährstoffe akquirieren, um wachsen und neue Gewebe erreichen zu können. Hierfür müssen die Pilze in der Lage sein, mit den vorhandenen Nährstoffen zurechtzukommen bzw. nicht vorhandene aber essentielle Metabolite *„de novo"* zu synthetisieren. Das Verständnis über die metabolischen Prozesse in der Infektionsphase kann dazu genutzt werden, um gezielt neue Wirkstoffe aufzufinden, die das Pilzwachstum negativ beeinflussen. Derzeit sind nur wenige Antimykotika zur Behandlung von invasiven Pilzerkrankungen vorhanden und deren Einsatz ist wegen starker Nebenwirkungen häufig nicht möglich. Daher erscheint es als dringende Notwendigkeit neue Wirkstoffe zu definieren, die spezifisch auf die pathogenen Pilze, nicht aber den Menschen wirken. Bisher ist jedoch weder im Detail bekannt, welche Nähstoffe in den infizierten Geweben nutzbar sind, noch ist bekannt, welche metabolischen Stoffwechselwege pathogene Pilze nutzen, um die vorhandenen Nährstoffe abzubauen.

Unsere Gruppe beschäftigt sich zur Zeit hauptsächlich mit den filamentösen Pilzen *Aspergillus fumigatus* und *Aspergillus terreus*, die ubiquitär verbreitet sind, eine hohe Sporulationskapazität besitzen und deren Sporen leicht durch die Luft verbreitet werden. Diese Sporen werden von immun-

ily spread by the air. Spores generally become inhaled deep into the lung by both, immunocompetent and immunosuppressed persons. In healthy people the defence mechanisms by alveolar macrophages and neutrophilic granulocytes are able to kill these spores early after inhalation, which prevents the development of an infection. However, since these defence mechanisms are either negatively affected or absent in immunosuppressed patients, a high risk for developing an invasive fungal infection is given.

We are, therefore, searching for essential fungal metabolic pathways in order to create a long lasting inhibition of fungal growth. For that purpose key enzymes of selected ana- and catabolic fungal pathways become purified and biochemically characterised to define their biochemical properties. This knowledge is essential for the development of antifungal compounds. To elucidate the impact of these enzymes during pathogenesis, we furthermore generate mutants of the fungi, which carry deletions in the respective genes. By this means it is possible to specifically investigate the impact of selected metabolic pathways for their contribution in virulence. We are, furthermore, in the process of optimising existing infection models and try to develop new models, which facilitate the screening for new virulence determinants and give a more reliable prediction on the impact of metabolic processes during different phases of the infection process. In addition, by modifying the immunosuppression regimen in infection models, we aim in a better understanding of the course of diseases caused by fungi.

supprimierten, wie auch gesunden Menschen tief in die Lunge inhaliert. In gesunden Menschen sind die Abwehrmechanismen über Makrophagen und neutrophile Granulozyten in der Lage die Sporen frühzeitig abzutöten, so dass es nicht zu einer Infektion kommt. Immunsupprimierte Personen, in denen diese Abwehrmechanismen nur unvollständig oder gar nicht funktionieren, sind einer erhöhten Infektionsgefahr ausgesetzt.

Wir suchen daher nach in der Pathogenese essentiellen Stoffwechselwegen, um das Wachstum der Pilze nachhaltig unterdrücken zu können. Hierbei werden Schlüsselenzyme ausgesuchter Abbau- und Synthesewege aufgereinigt und biochemisch charakterisiert, um deren katalytische Charakteristika definieren zu können, die für eine eventuelle Wirkstoffsuche wichtig sind. Um den Einfluss dieser Enzyme während der Pathogenese zu untersuchen, werden weiterhin Pilzmutanten hergestellt, die Deletionen der entsprechenden Gene tragen. Hierdurch kann gezielt untersucht werden, ob die ausgewählten Stoffwechselwege eine Bedeutung für die Virulenz der Pilze haben. Des weiteren versuchen wir Infektionsmodelle zu optimieren und neue Modelle zu entwickeln, die das Screening nach Virulenzdeterminanten vereinfacht und eine verlässlichere Aussage über die Bedeutung von metabolischen Prozessen in bestimmten Infektionsphasen ermöglicht. Weiterhin versuchen wir durch die Veränderung der Immunsuppression den Krankheitsverlauf besser zu verstehen.

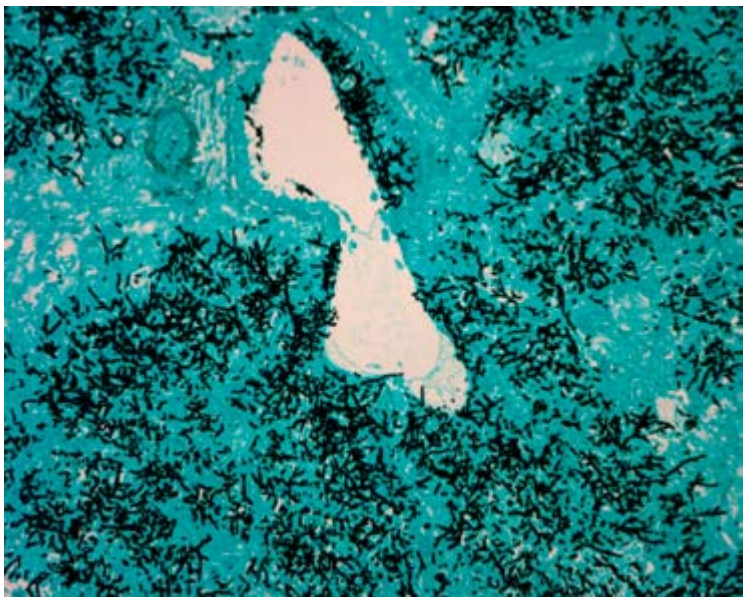


**Figure 1**
Invasive aspergillosis. The photograph shows the lung tissue of a mouse, which was immunosuppressed with corticosteroids prior to infection with *Aspergillus fumigatus* conidia. The black filaments represent the fungal mycelium after methenamine silver staining. The lung tissue appears blue/green.

## 1 Propionyl-CoA: A Toxic Compound!

Propionyl-CoA is a toxic compound, which is formed by degradation of propionate, odd chain fatty acids and the amino acids isoleucine, valine and methionine. The metabolite inhibits the pyruvate dehydrogenase complex, which is essential for shunting pyruvate from glycolysis into the tricarboxylic acid cycle (Krebs cycle). In addition, propionyl-CoA can inhibit other enzymes from various metabolic pathways, which deal on activated carboxylic acids. Humans metabolise propionyl-CoA via the so-called methylmalonyl-CoA pathway, which is characterised by the carboxylation of propionyl-CoA to methylmalonyl-CoA, an isomerisation and a rearrangement of the carbon skeleton *via* a radical mechanism to yield succinyl-CoA, a common intermediate of the Krebs cycle. The key enzyme in this pathway, the methylmalonyl-CoA mutase, contains the vitamin coenzyme $B_{12}$, which is essential for the generation of the radical in the enzymatic conversion of the substrate. Since fungi are neither able to synthesise coenzyme $B_{12}$ nor possess the methylmalonyl-CoA mutase, they have to convert propionyl-CoA by an alternative route. The fungal pathway involved in the degradation of propionyl-CoA is that via the methylcitrate cycle with the key enzyme methylcitrate synthase (Fig. 2). The methylcitrate synthase catalyses a condensation of propionyl-CoA with oxaloacetate forming methylcitrate and giving the pathway its name. A deletion of the methylcitrate synthase, therefore, leads to an inability to remove toxic propionyl-CoA. Such a deletion mutant accumulates high amounts of intracellular propionyl-CoA when grown in the presence of propionate (Fig. 3), odd chain fatty acids or selected amino acids, which in turn leads to strongly reduced growth rates.

We speculated that during invasive fungal growth proteins might become utilised as nutrients. Hydrolysis of proteins leads to the release of free amino acids. These amino acids

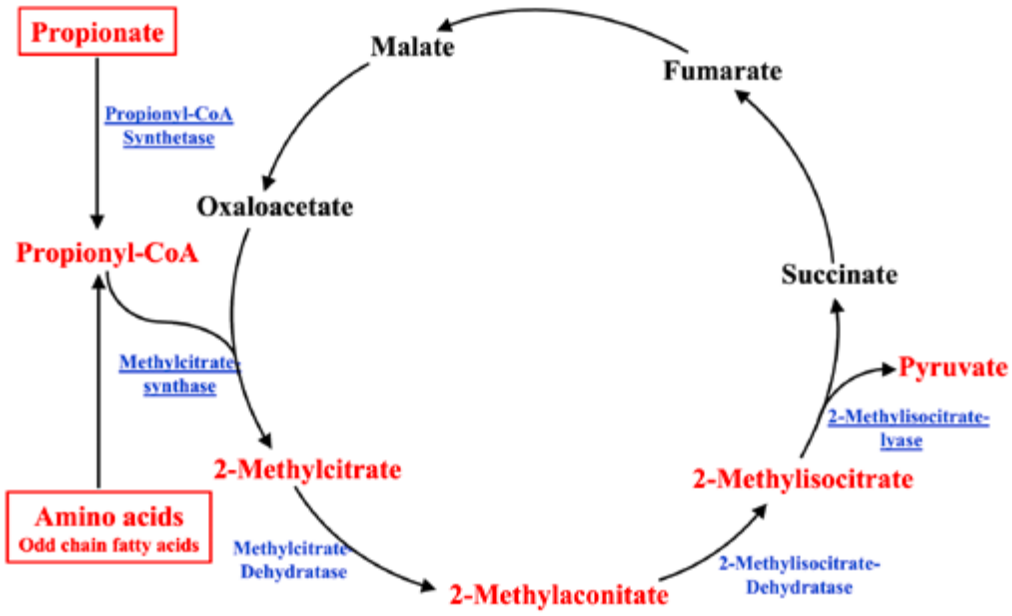


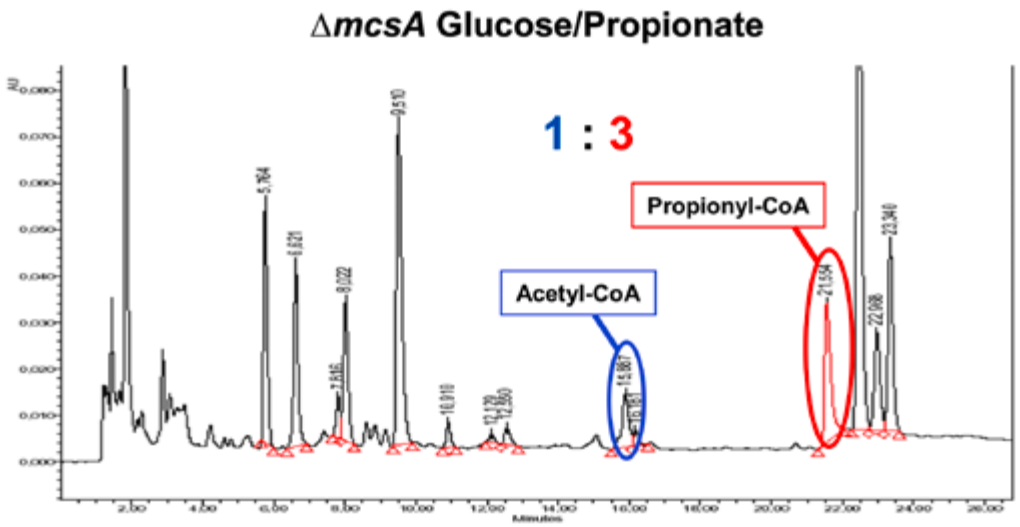

**Figure 2**
Scheme of the methylcitrate cycle. Propionyl-CoA is generated from direct activation of propionate or from the degradation of odd chain fatty acids or amino acids. A condensation reaction with oxaloacetate leads to methylcitrate. Further isomerisation reactions and final cleavage of methylisocitrate yields pyruvate and succinate. Pyruvate can be used for downstream metabolic processes.

can be taken up by the fungus and used for energy and biomass production. Among all 20 amino acids also isoleucine, methionine and valine will be taken up and, as described above, their degradation leads to the formation of propionyl-CoA, which was assumed to accumulate in a methylcitrate synthase deletion strain, thus self-poisoning the fungal cell. In order to proof this assumption we infected immunosuppressed animals with a wild-type strain and a methylcitrate synthase deletion strain, respectively, and followed both, survival of animals and fate of the fungal cells. Experiments revealed that the methylcitrate synthase mutant indeed was attenuated in virulence and animals, which survived the first phase of acute infection, were able to clear the fungal cells from the lungs. This result implicates that proteins are utilised during pathogenesis and showed furthermore that the methylcitrate cycle might act as a suitable target for the development of new antifungal drugs. Further experiments will show, whether the methylcitrate synthase is the only enzyme of the methylcitrate cycle, which may act as a suitable target for new antifungals or whether other enzymes of this cycle, such as methylcitrate dehydratase or methylisocitrate lyase, may also attenuate virulence and may provide additional targets to inhibit fungal growth during pathogenesis.

## 2 Lysine: An Essential Amino Acid?

Humans have to take up lysine from their food since they are not able to synthesise this amino acid *de novo*. In contrast, fungi possess a specialised pathway, the so-called alpha-aminoadipate pathway for the de novo synthesis of lysine. This pathway possesses some specific enzymes, which can only be found in fungi and a small number of bacteria. The key enzymes consist of the homocitrate synthase, which catalyses the condensation of acetyl-CoA and 2-oxoglutarate to form homocitrate,

**Figure 3**
HPLC analysis of a cytoplasmic extract of a methylcitrate synthase mutant (*ΔmcsA*) from *Aspergillus fumigatus*. The strain was grown on a mixture of glucose and propionate. The peaks for the CoA-esters acetyl-CoA and propionyl-CoA are highlighted. The ratio between acetyl-CoA and propionyl-CoA is 1 : 3, which is far above of that, which can be observed for a wild-type strain, denoting the inability of the mutant to remove the toxic propionyl-CoA.




the homoaconitase, which at least catalyses the reversible re- and dehydration of homoaconitate to homoisocitrate and the homoisocitrate dehydrogenase, which decarboxylates homoisocitrate into alpha-ketoadipate. In our studies, we aimed in the recombinant synthesis of these enzymes to study their biochemical parameters because former studies showed that a homoaconitase mutant is strongly attenuated in virulence, which implies that enzymes of the lysine biosynthesis might also act as suitable targets to fight against fungal infections. Currently we are focusing on the enzymatic catalysis performed by the homoaconitase because former studies revealed that homoisocitrate can be converted to homoaconitate, but it has never been shown that homocitrate can also be converted to homoaconitate, which would resemble the aconitase reaction from the Krebs cycle. Aconitases are able to dehydrate citrate to aconitate and to rehydrate aconitate to isocitrate. In the case that the homoaconitase can only perform the second part of the reaction, one important enzyme in the alpha-aminoadipate pathway of the lysine biosynthesis would remain unidentified. Purification of homoaconitase from *A. fumigatus* turned out to be difficult since enzyme levels were extremely low and the protein was unstable in the presence of air due to an iron sulphur cluster, which became oxidised in the presence of oxygen. Therefore, we produced the enzyme as a recombinant protein in *E. coli*. Although the enzyme was inactive in the first instance, we were able to reconstitute the iron sulphur cluster and obtained an active enzyme, which converted homoaconitate into homoisocitrate. However, preliminary analyses showed that no activity could be observed when homocitrate was used as a substrate, indicating that indeed a yet unidentified protein might be involved in the alpha-aminoadipate pathway. Therefore, we are now focusing on the identification of the unknown protein, which is required to complete the pathway.

In addition we started to perform virulence

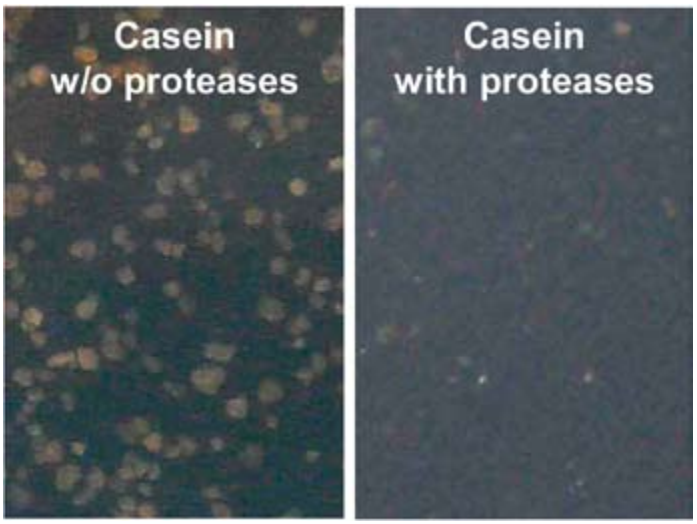



**Figure 4**
Hydrolysis of insoluble casein by proteases. The picture on the left side shows a solid growth medium, which contains insoluble casein as nutrients. The casein is visible as granules within the medium. The right side shows the same medium after treatment with proteases from *Aspergillus fumigatus*. The proteases were partially purified from a culture supernatant by ammonium sulphate precipitation and plated on the medium. The picture was taken after an incubation time of 24 h. Most of the casein granules had disappeared, indicating the hydrolysis of the protein.

studies with an *A. fumigatus* homocitrate synthase deletion mutant, which requires lysine for growth. In this study we are investigating, whether the mutant is able to germinate from spores after reaching the lung tissue. This ability would indicate that free lysine is present in sufficient concentrations within infected tissues to support fungal growth. The inability to germinate would, in turn, indicate that lysine is only present in proteins, which would require the action of proteases to release lysine and allow fungal growth. The ability of proteases to hydrolyse even the insoluble form of casein, an important protein in milk, is shown in figure 4, in which casein granules disappear after treatment with proteases purified from *A. fumigatus* culture supernatants. Since these proteases are assumed to be produced only from hyphae but not conidia, the early inhibition of lysine biosynthesis could provide a useful prophylactic tool to prevent immunosuppressed patients from invasive fungal infections. Therefore, the information on the growth behaviour of such a mutant during pathogenesis is required to evaluate, whether the lysine biosynthesis pathway could indeed provide antifungal drug targets.

## 3 The Conundrum on the Isocitrate Lyase

Isocitrate lyase is a key enzyme of the glyoxylate cycle and required for the *de novo* synthesis of oxaloacetate when microorganism are growing on C2-generating carbon sources such as ethanol, acetate, fatty acids or lipids. Former studies revealed that the pathogenic fungus *Candida albicans* requires a functional isocitrate lyase for full virulence. Since the enzyme is highly abundant when cells are confronted with immune effectors and is also produced during epithelium invasion and within different tissues, it was concluded that *C. albicans* is highly dependent on lipid metabolism during pathogenesis. In order to

analyse, whether isocitrate lyase is of general importance for fungal virulence, we generated an isocitrate lyase deletion mutant of *A. fumigatus* and tested the strain for its growth behaviour under different *in vitro* and *in vivo* conditions. As expected, the mutant was unable to grow on acetate, ethanol, fatty acids or lipids, but grew normal on media, which did not require the glyoxylate cycle such as glucose, glycerol or amino acids. Unexpectedly, the mutant strain displayed no attenuation in virulence when tested in a murine model for invasive aspergillosis, although isocitrate lyase was detected in germinating conidia phagocytosed by macrophages. In addition, invasive growth within the lung tissue was not affected, implicating that the glyoxylate cycle is only of minor importance for the pathogenesis of *A. fumigatus*. Nevertheless, lipid metabolism is not dependent on the glyoxylate cycle, when alternative carbon sources such as proteins are present. An isocitrate lyase mutant metabolised lipids in the presence of peptone and was indistinguishable from a wild-type strain on such a medium. Therefore, our studies clearly showed that lipids do not provide the sole carbon source during pathogenesis of *A. fumigatus* but it might provide one carbon source amongst others. Since the isocitrate lyase mutant from *C. albicans* was, as mentioned above, strongly attenuated in virulence, two possibilities are conceivable: (a) *C. albicans* is strongly dependent on the metabolism of lipids or other C2-generating carbon sources and starves in the absence of a functional glyoxylate cycle or (b) the glyoxylate cycle from *C. albicans* is not only required for the *de novo* synthesis of oxaloacetate during growth on C2-generating carbon sources but is also required for other metabolic processes. Therefore, we started to re-investigate the regulation of the glyoxylate cycle from *C. albicans*. In case the glyoxylate cycle is only important during growth on C2-generating carbon sources, it can be assumed that different fungi use different nutrients during pathogenesis. This in turn would indicate that it might become difficult to find metabolic pathways, which act as universal targets for antifungal drug development. In case that the regulation of the isocitrate lyase is not dependent on C2-generating carbon sources in *C. albicans* but important on various nutrients, the question, which nutrients are used during pathogenesis would remain open. Further studies will then be required to identify the metabolic pathways essential during pathogenesis of this fungus.

## Group members

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## External funding

Deutsche Forschungsgemeinschaft
Schwerpunktprogramm 1160: Kolonisation und Infektion durch humanpathogene Pilze
Teilprojekt: Metabolism and morphogenesis of human pathogenic fungi
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Deutsche Forschungsgemeinschaft
Excellence Graduate School JSMC – Jena School for Microbial Communication
Teilprojekt: Interaction of *Candida albicans* and host cells: Fungal nutrient acquisition and host defense mechanisms
Matthias Brock

Deutsche Forschungsgemeinschaft
Biochemie des humanpathogenen Pilzes *Aspergillus fumigatus*: Untersuchung der Interaktion des Acetat-und Propionatstoffwechsels
Matthias Brock

## Selected publications

(HKI authors in bold)


Ebel F, Schwienbacher M, Beyer J, Heesemann J, **Brakhage AA**, **Brock M** (2006) Analysis of the regulation, expression, and localisation of the isocitrate lyase from *Aspergillus fumigatus*, a potential target for antifungal drug development. *Fungal Genet Biol* 43, 476-489.

**Sugareva V**, **Härtl A**, **Brock M**, Hubner K, Rohde M, **Heinekamp T**, **Brakhage AA** (2006) Characterisation of the laccase-encoding gene abr2 of the dihydroxynaphthalene-like melanin gene cluster of *Aspergillus fumigatus*. *Arch Microbiol* 186, 345-355.

**Behnsen J**, Narang P, Hasenberg M, Gunzer F, Bilitewski U, Klippel N, Rohde M, **Brock M**, **Brakhage AA**, Gunzer M (2007) Environmental dimensionality controls the interaction of phagocytes with the pathogenic fungi *Aspergillus fumigatus* and *Candida albicans*. *PLoS Pathog* 3, e13.

**Schöbel F**, Ibrahim-Granet O, Avé P, Latgé J-P, **Brakhage AA**, **Brock M** (2007) *Aspergillus fumigatus* does not require fatty acid metabolism via isocitrate lyase for development of invasive aspergillosis. *Infect Immun* 75, 1237-1244.


## Collaborations

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## Bio Pilot Plant

# Bio Pilot Plant

The research of the HKI Bio Pilot Plant is mainly focused on the development, optimization and scale-up of biotechnological processes from flask cultures via laboratory scale to pilot scale.

The team of the HKI Bio Pilot Plant is experienced in strain improvement, cultivation and fermentation of a large variety of prokaryotic and eukaroytic microorganisms. This includes genetic engineering of strains to optimize their productivity and to monitor specific physiological parameters during fermentation processes.

State-of-the-art analytical methods as well as techniques and equipment for downstream processing and purification of low molecular weight natural products, biopolymers and proteins are available.

Our research strategy is characterized by a highly interdisciplinary approach closely linking microbial physiology, genetic engineering and bioprocess engineering. Experimental and theoretical methods are combined to analyse and control high performance fermentations considering the physiological requirements of the producing microorganisms.

## INTRODUCTION | EINLEITUNG

**Head:**
**Dr. Uwe Horn**

Forschungsschwerpunkte des Biotechnikums sind die Entwicklung und Optimierung biotechnologischer Prozesse von Naturstoff-Produzenten. Das reicht von der Petrischale über den Labormaßstab bis zum Pilotmaßstab. Dazu stehen Fermenter von bis zu 3.000 l Nettovolumen sowie entsprechend großvolumige Aufarbeitungsmöglichkeiten zur Verfügung.

Wir entwickeln außerdem neue Verfahren zur Kultivierung von Bakterien und Pilzen und für die Herstellung von Proteinen durch die so genannte Hochzelldichte-Fermentation. Damit lassen sich sehr kompakt und schnell Proteine produzieren.

Um die Anforderungen der produzierenden Mikroorganismen zu analysieren und bei der Fermentation zu steuern, werden experimentelle und theoretische Methoden kombiniert. Das Biotechnikum arbeitet dabei intensiv mit den Forschungsabteilungen des HKI sowie Gruppen der Friedrich-Schiller-Universität Jena und der FH Jena zusammen.

# Scientific Projects

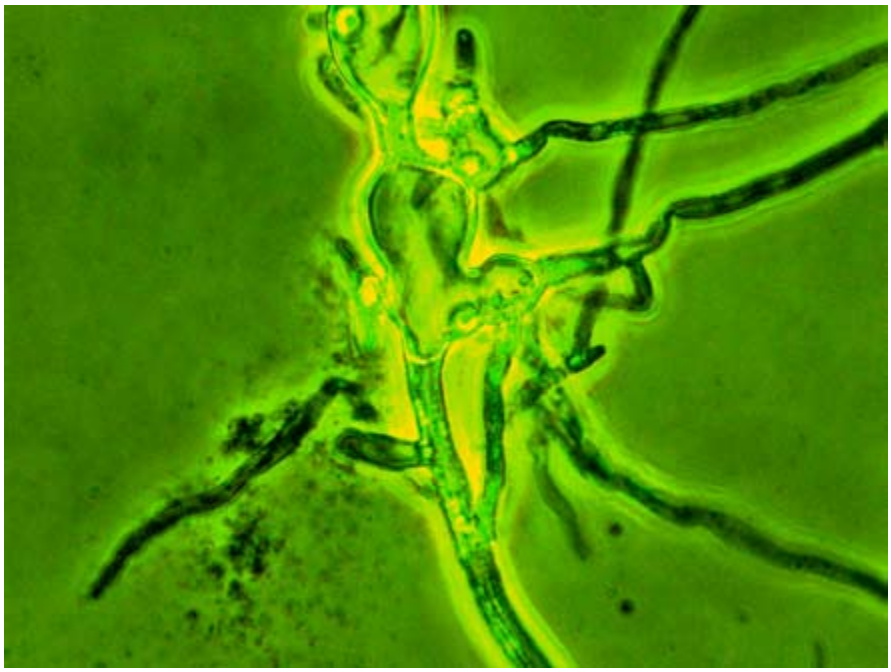

**Figure 1**
Light microscope imaging of *Blakeslea* sp.

## 1 Production of Microbial Biomass and Microbial Natural Products within the Internal Product Line, for Research Projects of HKI Groups and External Academic Partners

Group Leaders: Klaus-Dieter Menzel, Gundela Peschel, Martin Roth, Uwe Horn

A large number of microbial products from different microorganisms were produced within the scope of the Internal Product Line (IPL) and for partners from academia. 530 fermentations at the 1 to 300 liter scale were performed within 2006 – 2007 including preparation of inoculum cultures, fermentation and monitoring of the fermentations by examination of samples and data acquisition. Downstream processing has been performed: Separation of supernatant and biomass, fractionation of supernatant by preparative HPLC or adsorption on synthetic resins, solvent extraction, and concentration and freeze drying of the extracts. In 2006 – 2007 products from about 20,000 liter fermentation broth were prepared.

### Collaborations within the IPL of HKI and with External Academic Partners

(1) Microbial interactions - isolation and characterization of novel rhizoxin derivatives produced by bacterial endosymbionts isolated from *Rhizopus microsporus* strains (HKI, Dept. Biomolecular Chemistry)

**Activities at Pilot Plant:**

Media optimisation and process development for cultivation of endosymbiontic bacteria belonging to the genus *Burkholderia*.

Pilot scale fermentation of *Burkholderia* strains and downstream processing to produce rhizoxin derivatives.

(2) Natural products screening of diverse microorganisms isolated from special habitats (HKI, Dept. Biomolecular Chemistry)

**Activities at Pilot Plant:**
Pilot scale fermentations (30 to 300 L) of microorganisms (extremophilic and rare actinomycetes, actinomycetes from heavy metal contaminated habitats, fungi) to isolate and produce new bioactive natural products.
Downstream processing, especially preparative HPLC, to isolate new bioactive substances.

(3) Engineered biosynthesis of aromatic polyketides (HKI, Dept. Biomolecular Chemistry)
**Activities at Pilot Plant:**
Pilot scale fermentation of recombinant *Streptomyces* strains and downstream processing to purify aureothin derivatives.

(4) Molecular physiology of sexual and parasitic differentiation in zygomycetes (University Jena, Institute of Microbiology, General Microbiology and Microbial Genetics)
**Activities at Pilot Plant:**
Improvement of the pilot scale fermentation process for *Blakeslea* sp. to produce trisporic acid derivatives.

(5) Potential use of heavy metal resistant streptomycetes in bioremediation (University Jena, Institute of Microbiology, Microbial Phytopathology)
**Activities at Pilot Plant:**
Pilot scale fermentation of heavy metal resistant *Streptomyces* strains to produce biomass for bioremediation studies at the former uranium mining site in Eastern Thuringia.

(6) Selection and expression of VHH-antibodies against surface antigens of *Aspergillus fumigatus* (HKI, Dept. Molecular and Applied Microbiology)
**Activities at Pilot Plant:**
Supply of the camelid antibody library. Assistance with the selection of the recombinant antibodies, fermentation and purification of the recombinant antibodies.

(7) Fermentation and purification of SAP-Proteases using *Pichia pastoris* (HKI, Dept. Molecular and Applied Microbiology)
**Activities at Pilot Plant:**
Assistance with fermentation and purification of SAP-Proteases using *Pichia pastoris*.

## 2 Selection and Characterization of Camelid VHH-domains from a Native and Fully Synthetic Library: Directed Selection of a Conformational Antibody Domain that Prevents Mature Amyloid Fibril Formation

Group Leaders: Gernot Habicht, Uwe Horn

The selection of conformation-sensitive binders based on a native and fully synthetic library of camelid VHH-domains. VHH-domains are the terminal immunoglobulin domains of heavy chain antibodies from *camelidae* (camels, dromedars etc.). These antibodies do not contain any light chains and only the heavy chain, i.e. the VHH-domain, mediates antigen binding. Selection from recombinant libraries provides several advantages. It enables generation of binders to antigens for which self-tolerance may prevent antibody formation or which are unstable during vaccination. It enables application of a competitive selection regime, and selected binders are available in large quantities and readily amenable for genetic manipulation. Using this technology we have generated the conformation-sensitive VHH-domain B10 that recognizes specifically amyloid fibrils formed from Alzheimer's Aβ peptide but not the freshly dissolved, putatively monomeric peptide.
B10 was raised against the structure of a mature amyloid fibril morphology formed from Alzheimer's Aβ (1-40) peptide. The problem of selecting simply sequence-specific VHH-domains and not conformation-sensitive ones was minimised by addition of a tenfold molar excess of disaggregated Aβ (1-40) peptide to the supernatant. Therefore, sequence-specific VHH-domains that cross-react with disaggregated Aβ peptide can be removed readily.
Surface plasmon resonance measures an ap-

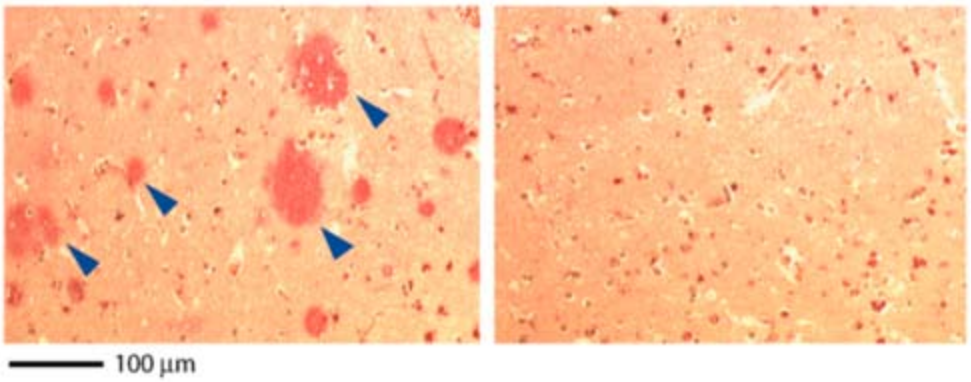


**Figure 2**
Hippocampal sections from an Alzheimer's disease patient (left) or a control case without clinical signs of dementia (right) stained with B10AP and visualised by "Permanent Red" chromogene.

parent dissociation constant ($K_D$) of 475 ± 54 nM. Genetic fusion of B10 to alkaline phosphatase, a naturally dimeric protein, leads to a fusion protein, termed B10AP, that is also dimeric. The associated bivalent antigen binding characteristics increase the fibril affinity to an apparent $K_D$ of 7.22 ± 0.97 nM. Neither B10AP nor B10 show significant interactions with disaggregated Aβ (1-40) peptide.

The phosphatase moiety of B10AP facilitates applications in immunoblots or immunohistochemistry. Spotted onto nitrocellulose, Aβ (1-40) amyloid fibrils can be detected in quantities of 50 ng or more. Hippocampal sections from confirmed cases of Alzheimer's disease probed with B10AP show clear plaque staining. The plaques recognised in these sections possess diameters of at least 10 µm. Eleven out of twelve confirmed cases of Alzheimer's disease show plaque-positive staining with B10AP, while none of our 10 age-matched controls shows a similar staining. B10AP detects also cell-culture derived Aβ plaques, and double staining with a sequence-specific anti-Aβ antibody shows intensive colocalisation for large plaques, while small Aβ assemblies produce a more heterogeneous staining. These data establish firmly that the B10-epitope is conformationally specific for amyloid fibrils and that recombinant libraries can be utilised successfully for the purpose of generation of such conformation-sensitive binders.

**In collaboration**
with Peter Hortschansky, Dept. Molecular and Applied Microbiology, HKI and the Junior Research Group of Marcus Fändrich, FLI Jena

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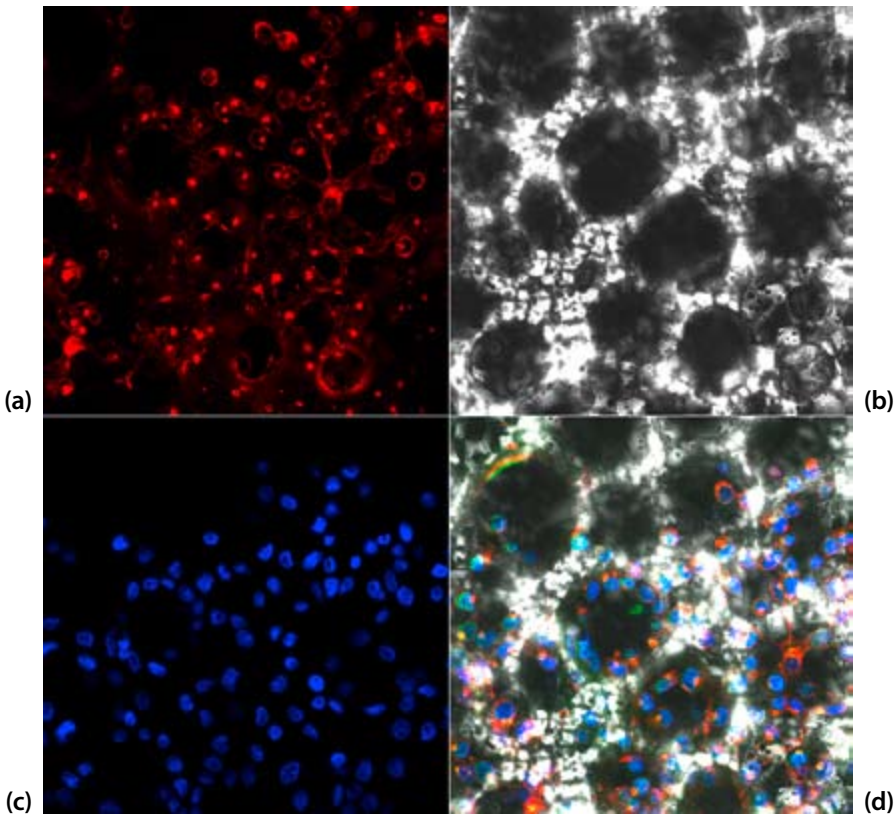
Proc Natl Acad Sci USA, 2007, 104(49): 19232–19237

**Figure 3**
CLSM micrographs of HaCaT cells after culture for 72 h on films of P(4HB)/CH Staining with Hoechst 33342 and Alexa Fluor 594 conjugated wheat-germ agglutinin (Molecular Probes/Invitrogen) was used to visualize cell nuclei (blue) and cell membranes (red). Chitosan at the pore rims in (a) is also visualized in red.




## 3 Polyhydroxyalkanoates (PHA) as Biodegradable and Biocompatible Materials
## Growth of Keratinocytes on Porous Films of Poly(3-hydroxybutyrate) and Poly(4-hydroxybutyrate) Blended with Hyaluronic Acid and Chitosan

Group Leaders: Gundela Peschel, Anke Konrad, Peter-Jürgen Mueller, Martin Roth

In collaboration with Tepha, Inc. (Lexington, MA., USA) polyhydroxyalkanoates (PHA) have been studied as biodegradable and biocompatible materials for use in medical devices and tissue engineering scaffolds. The adherence and proliferation of cells on a scaffold is influenced by surface conditions including chemical (toxicity) and physical properties (hydrophilicity, charge) and surface topography (porosity, roughness). Biocompatibility comprises all these properties of the material. Non-porous films of poly(3-hydroxybutyrate) [P(3HB)] and poly(4-hydroxybutyrate) [P(4HB)] as well as porous films of these PHA`s blended with HA (hyaluronic acid) or chitosan (CH) were used in the proliferation studies with HaCaT keratinocytes in comparison with commercially available films of HA benzylester and a polylactic acid copolymer.

Surprisingly on the non-porous, very smooth P(4HB) film a fivefold higher cell count compared with the P(3HB) film was detected. In addition, cells formed numerous filaments referring to a good biocompatibility of the material.

Cells can obviously better proliferate on rough or porous scaffolds. There is an influence of pore size and pore number on cell proliferation. A high number of pores and a varying pore size of smaller (ca. 50 μm) and larger pores (up to 150 μm) as in P(4HB)/HA seems to be optimal for attachment and proliferation of keratinocytes. From SEM-images or CLSM images we get more information about the influ-

**In collaboration**
with H.-M. Dahse, Dept. Infection Biology, HKI, G.D. Wieland, Dept. Infection Biology, HKI, D.P. Martin, Tepha, Inc., USA

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ence of the surface structure of porous films on the attachment and growth of cells. Cell walls and nuclei were stained with special dyes and pseudocoloured in red and blue, respectively. The structure of films and the attached cells were shown by digital interference contrast images additionally. The overlay of the different images shows that the cells grow preferentially at the rims of pores.

On the porous composite films the maximum cell number is reached already after 72 h incubation. The order of maximum cell numbers was P(4HB)/HA > P(4HB)/CH > P(3HB)/HA > P(3HB)/CH > P(3HB)/pectin > P(3HB)/alginic acid. A slightly inhibiting influence of CH on cell proliferation cannot be excluded.

The porous P(4HB) and P(3HB) composite films have excellent surface properties as demonstrated by the famous growth of the keratinocyte cell line HaCaT and appear suitable for use as epidermal grafts. The porous surface supports the proliferation of HaCaT cells. The cells preferentially attach to the rims of pores. The material properties of P(4HB) obviously promote cell growth additionally, as indicated by the high proliferation rate and the numerous filaments of cells.

Soft porous P(4HB) composite films retain the high flexibility and ductility of pure P(4HB) films. These fact in association with the excellent biocompatibility let porous P(4HB) appear suitable for use as patches in surgery too. In contrast, porous P(3HB) composite films have a lower tensile strength and are less ductile. Nevertheless they may still be suitable as wound dressings because the films are flexible enough to fit to the skin.

## 4 Enhanced Expression of Heterologous Proteins in *Escherichia coli* A Dual Expression Platform to Optimize the Soluble Production of Heterologous Proteins in the Periplasm of *Escherichia coli*

Group Leaders: Mario Kraft, Uwe Knüpfer, André Kacholdt, Uwe Horn

The functional analysis of individual proteins or of multi-protein complexes – since the completion of several genome sequencing projects – is in focus of current scientific work. Many heterologous proteins contain disulfide-bonds, required for their correct folding and activity and therefore, need to be transported to the periplasm. The production of soluble and functional protein in the periplasm often needs target-specific regulatory genetic elements, leader peptides and folding regimes. Usually the optimization of periplasmic expression is a step-wise and time-consuming procedure.

To overcome this problem we developed a dual expression system, containing a *degP*-promoter based reporter system and a highly versatile plasmid set. This combines the differential protein expression with the selection of a target-specific expression plasmid.

For the validation of this expression tool two different molecular formats of a recombinant antibody directed to the human EGF-receptor and human 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2) were used. By application of this expression system we demonstrated that the amount of functional protein is inversely proportional to the on-line luciferase signal. We showed that this technology offers a simple tool to evaluate and improve the yield of functionally expressed proteins in the periplasm, which depends on the used regulatory elements and folding strategies.

Kinetic studies of protein misfolding by on-line monitoring were performed during expression of two different molecular formats of a recombinant miniantibody specific to the human EGF-receptor (EGFR). For the non-induced cultures, during the whole cultivation only a low basal level of about 40 relative luminescence units (RLU) was determined. In contrast to this we obtained a strong increase of the luciferase signal 2 h after protein induction, corresponding to the product formation kinetic of the miniantibodies. This resulted in a final level of appr. 500 and 300 RLU.

To determine the influence of folding promoting agents on the ratio of misfolded miniantibodies we investigated two time dependent feeding strategies on the expression of the scFvdhlx miniantibody construct. The luciferase signal reached only the basal level when feeding was started at the beginning of the cultivation. However, when the feeding solu-

**In collaboration**
with Rolf Wenderoth Dept. Molecular and Applied Microbiology, HKI and Patricia Pietschmann and Björn Hock, Merck KgaA, Darmstadt

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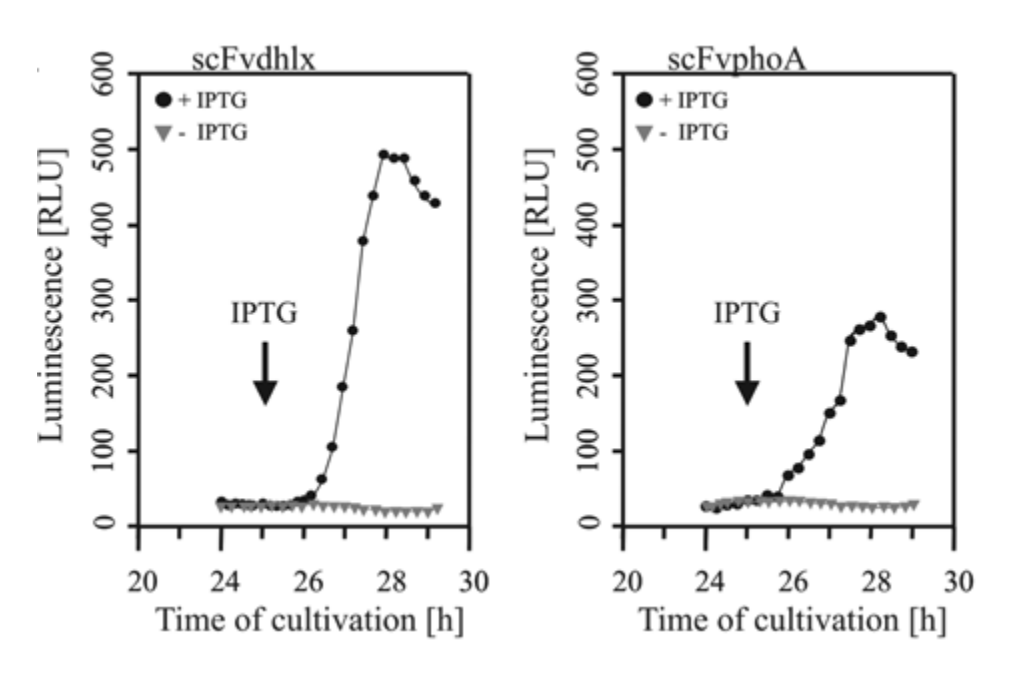
**Figure 4**
Induction of luciferase reporter gene after expression of miniantibodies under non-inducing and inducing conditions (left) and after co-expression of the molecular chaperon *Skp* (right).




tion was added 2 h after the induction of the scFvdhlx miniantibody, there was an increase in the luciferase signal comparable to the induced cultivation without any feeding, followed by a constant level of about 170 RLU up to the end of the cultivation time. Based on these results protein folding of the second antibody format scFvphoA was investigated by adding sorbitol and betaine at the beginning of the cultivation only. We obtained a slight increase of the luciferase signal, comparable with the first antibody format scFvdhlx. In addition, we analyzed the influence of co-expression of the periplasmic chaperone Skp on soluble miniantibody expression. For this purpose we inserted the periplasmic chaperone Skp into the expression plasmids. The Skp co-expression experiment showed for both antibody formats a lower increase of the luciferase gradient with a maximal luciferase activity of approximately 200 RLU.

To achieve differential periplasmic expression of heterologous proteins we developed a highly versatile plasmid set containing 36 plasmids. We used three different efficient derivatives of the *lacZ*-promoter, as well as three ribosome-binding sites (RBS): (i) the highly efficient RBS_T7g10, (ii) the RBS_lac and (iii) a synthetic RBS (RBS_Var3). Four different leader peptides were used to transport the protein into the periplasm: (i) *E. coli* maltose-binding protein (MalE), (ii) *E. coli* outer membrane protein A (OmpA), (iii) *E. coli* alkaline phosphatase (PhoA) (Monteilhet et al. 1993), and (iv) *Erwinia carotovora* pectatlyase B (PelB).

As shown by the luminescence assay, differential expression of scFvphoA as well as 11β-HSD2 resulted in the highest luminescence levels, when the strongest promoter (CTU) or RBS (RBS_T7g10) was used. In contrast, expression of the host protein MBP (control) resulted only in luminescence background level activity. In comparison to the expression in the plasmid pCUlac_oA, production of miniantibodies in plasmids like pCUT7_pA, pCTUlac_mE, and pCTUlac_pA resulted in an approximately 10-

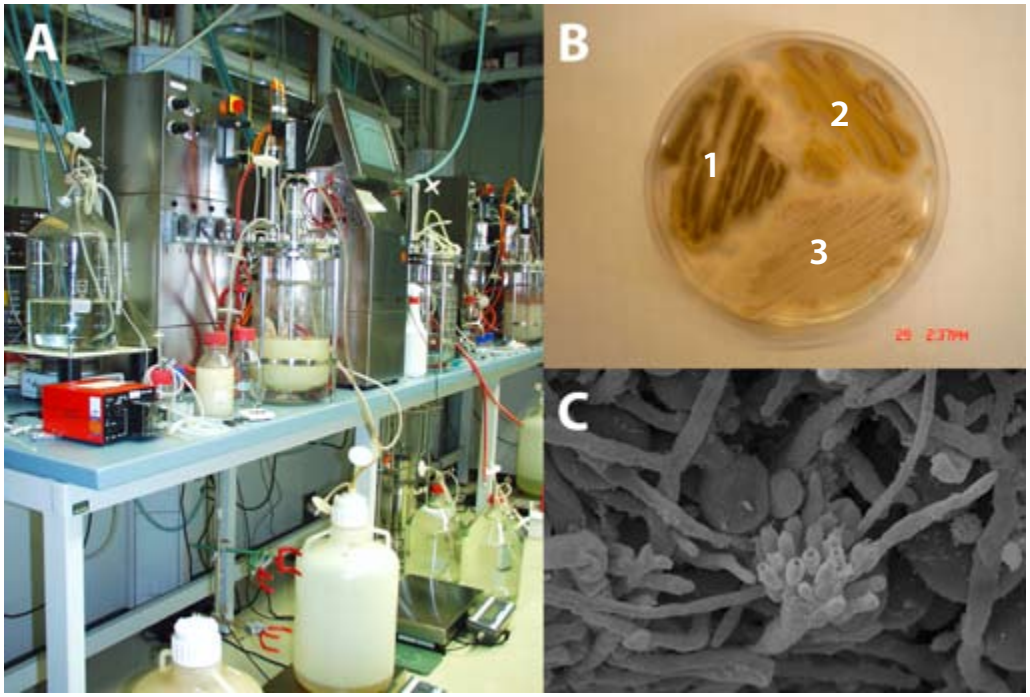



**Figure 4**
(A) Continuous cultivation apparat. (B) *Aspergillus nidulans* grown on mineral medium agar, {1} green colored spores of Wild type strain, {2} Yellow colored spores of a strain with putative PKS gene replaced by G-Luc, {3} White colored spores of the strain where PKS responsible for spore color WA is replaced by G-Luc (C) SEM picture showing conidiophore obtained by continuous cultivation, scale bar 1cm=2µm.

fold increase of the luminescence level. Unlike the other leader peptides, OmpA directed translocation of miniantibody fusion resulted in the lowest luminescence level independent of the genetic element used.

Based on results from plate cultivation several clones were selected for scale-up expression. However, the amount of scFvphoA under control of pCUT7_mE was quite low with approximately 3.5 mg/g dry cell weight (X), whereas expression using the pCTUT7_pB or pCUlac_oA plasmids showed a 6- or 7 fold increase of soluble miniantibody. As indicated by ELISA, accumulation of soluble 11β-HSD2 in the periplasm was up to 8-fold increased using the expression plasmids pCUlac_pA or pCUlac_pB in contrast to the expression sample pCUT7_mE or pCT7_pB.

Utilization of the leader peptides of PhoA and PelB resulted in increased accumulation of soluble 11β-HSD2, caused by improved translocation through the inner membrane. ELISA analysis showed that the yield of soluble 11β-HSD2 was approximately 1.5-3-fold higher when expression was mediated under control of plasmids like pCUlac_pA and pCUlac_pB compared to plasmids with the same regulatory genetic elements. For both proteins, expression or transport controlled by protein-specific regulatory genetic elements as well as by leader peptides yielded an increased accumulation of soluble protein.

## 5 Regulation of Secondary Metabolite Gene Expression in *Aspergillus nidulans*

Group Leaders: Anindita Sarkar, Alexander N. Funk, Uwe Horn

The genome sequencing of the organisms has thrown open more possibilities for secondary metabolite discovery. In the genetic model organism *Aspergillus nidulans*, approximately 30 putative secondary metabolite gene clusters

**In collaboration**
with Axel Brakhage, Dept. Molecular and Applied Microbiology, HKI, Kirstin Scherlach, Dept. Biomolecular Chemistry, HKI, Christian Hertweck, Dept. Biomolecular Chemistry, HKI.

have been identified, however only a handful of metabolites have been discovered and affiliated to the gene clusters. This suggests that most of the secondary metabolite gene clusters are silent and under specific or epigenetic control. Identification of the terrequinone cluster by deletion of a global regulator gene *lae* (Shwab et al, 2007) and aspoquinolone cluster by over-expressing a specific transcriptional factor (Bergmann et al, 2007) are few examples supporting the view.

### Secondary metabolite gene expression studies

The transient expression and weak promoter strength are the greatest challenges in the secondary metabolite gene expression studies. Reporter systems such as *Gaussia* Luciferase from *Gaussia princeps*, exhibit flash kinetics i.e. high intensity signal is generated and is decayed with time. Recombinant *Aspergillus nidulans* with putative polyketide synthase gene promoter fused to *Gaussia* luciferase were generated. The fungus was cultivated in continuous cultivation scheme under various nutrient limitation regimes e.g. C, N & P limitation and different growth rates (0.2/hr, 0.1/hr & 0.05/hr). By monitoring the reporter activity in the recombinant fungus, the time window of expression of the polyketide synthase gene was delineated. We showed that the reporter gene is active under transient growth phase.

### Fine tuning of the reporter system

Next task was to enhance the extra-cellular G-Luc detection. It was assumed that fusion with native protein would firstly, protect the secreted luciferase from the *Aspergillus* extra-cellular protease activity and secondly, would increase the secretion efficiency and thus enhance the extra-cellular secretion. Hence, *Gaussia* luciferase under the controllable alcAp promoter was fused to native Gluco-amylase. However experimentally, gluco-amylase fusion seemed to have an adverse effect on luciferase secretion and detection.

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Alexander Thümmler (until 11/2006)
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Elisabeth Zentgraf (until 03/2006)

## External funding

Deutsche Forschungsgemeinschaft
Excellence Graduate School JSMC –
Jena School for Microbial Communication
Teilprojekt: Influence of the amino acid metabolism and stress responses during the induction phase of *Escherichia coli* for the production of recombinant proteins – measuring the signal exchange during high cell density fermentation
Uwe Horn

Bundesministerium für Bildung und Forschung
SERIZELL – Hochdurchsatz-Bioassay-System auf Basis mikroserieller Zellkulturen in flüssig/flüssig-Zweiphasensystemen
Teilprojekt: Systemevaluierung mit ausgewählten Bioassays
Martin Roth

Bundesministerium für Wirtschaft und Technologie
Fördermaßnahme PRO INNO
Entwicklung innovativer Methoden zur kontinuierlichen Simultanfermentation cofaktorabhängiger, rekombinanter Enzyme; Entwicklung eines rekombinanten Wirt-Vektor-Systems für die Biosynthese von 10-Deacetylbaccatin III-10-O-Acetyltransferase
Karsten Willing

Industry
Tepha Inc., Cambridge (USA)
Improvement and Scaling-up of Fermentations to Produce Polyhydroxyalkanoates
Martin Roth

Industry
Merck KGaA, Darmstadt
Expression rekombinanter Glukose Dehydrogenase in *Escherichia coli* im Pilot-Maßstab und dessen Reproduzierbarkeitsprüfung
Uwe Horn

Industry
Shogoo Pharmaceuticals AG, Basel, Switzerland
Verfahrensentwicklung und Verfahrensoptimierung für die biotechnologische Herstellung eines antifungalen Antibiotikums
Martin Roth

Industry
Biosynergy GmbH, Leipzig
Stammhaltung und Stammpflege von *Blakeslea trispora* KR 74(+) und KR 86 (-) und die Prüfung des β-Carotinsynthesepotentials
Arnulf Christner

Industry
N-Zyme BioTec GmbH
Fermentation von Streptomyceten zur Gewinnung extrazellulärer Enzyme und deren nachfolgende Aufbereitung
Martin Roth / Uwe Horn

## Selected publications

(HKI authors in bold)


Gellermann GP, Ullrich K, Tannert A, Unger C, **Habicht G**, **Hortschansky P**, Sauter S, **Horn U**, **Möllmann U**, Decker M, Lehmann J, Fändrich M (2006) Alzheimer's plaque formation in a human macrophage system to reveal the inhibitory potential of different drugs. *J Mol Biol* 360, 251-257.

**Winkler R**, **Richter MEA**, **Knüpfer U**, Merten D, **Hertweck C** (2006) Regio- and Chemoselective Enzymatic *N*-Oxygenation *In Vivo*, *In Vitro*, and *in Flow*. *Angew Chem Int Ed* 45, 8016-8018.

**Habicht G**, Haupt C, Friedrich RP, **Hortschansky P**, Sachse C, Meinhardt J, Wieligmann K, Gellermann GP, Brodhun M, Götz J, Halbhuber K-J, Röcken C, **Horn U**, Fändrich M (2007) Directed selection of a conformational antibody domain that prevents mature amyloid fibril formation by stabilizing Aβ protofibrils. *Proc Natl Acad Sci USA* 104, 19232-19237.

**Kraft M**, **Knüpfer U**, **Wenderoth R**, Pietschmann P, Hock B, **Horn U** (2007) An online-monitoring system based on a synthetic sigma32 dependent tandem promoter for visualization of misfolded proteins in the cytoplasm of *Escherichia coli*. *Appl Microbiol Biotechnol* 75, 397-406.

**Partida-Martinez LP**, **de Looß CF**, **Ishida K**, **Ishida M**, **Roth M**, Buder K, **Hertweck C** (2007) Rhizonin, the first mycotoxin isolated from the zygomycota, is not a fungal metabolite but is produced by bacterial endosymbionts. *Appl Enrivon Microbiol* 73, 793-797.

**Peschel G**, **Dahse H-M**, **Konrad A**, **Wieland GD**, **Mueller P-J**, Martin DP, **Roth M** (2008) Growth of keratinocytes on porous films of poly(3-hydroxybutyrate) and poly(4-hydroxybutyrate) blended with hyaluronic acid and chitosan. *J Biomed Mat Res A* 85, 1072-1080.


## Collaborations

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Institut für Physikalische Hochtechnologie, Jena
Dr. T. Henkel

Technische Universität Ilmenau
Prof. Dr. M. Köhler

Friedrich-Schiller-Universität Jena
Prof. Dr. E. Kothe, PD Dr. C. Schimek,
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EMC microcollections GmbH, Tübingen

Evotec Technologies GmbH, Hamburg

Impuls GmbH, München

Jenoptik Mikrotechnik GmbH, Jena

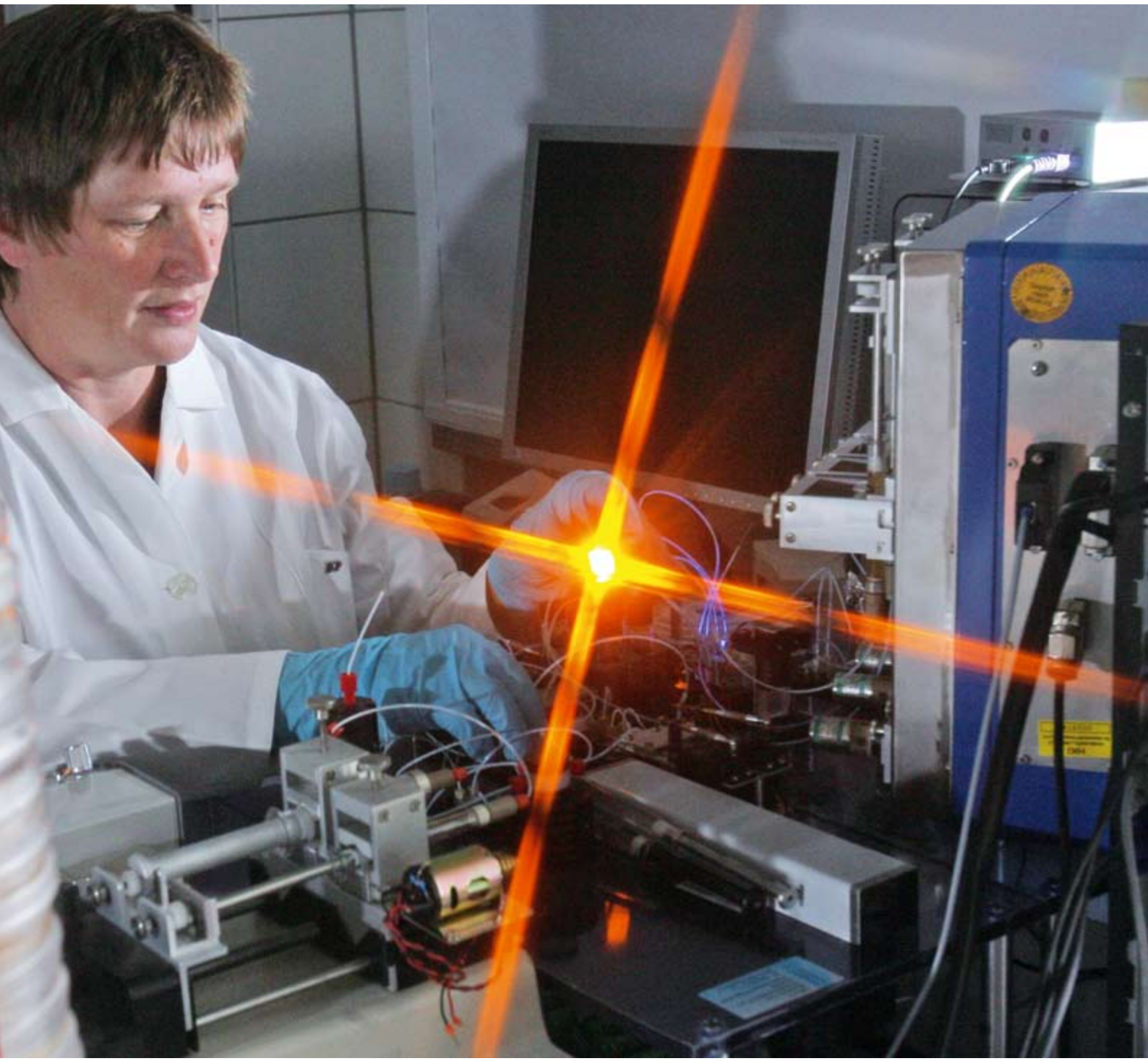
KreActiv GmbH Jena

Merck KgaA, Darmstadt

Tepha, Inc. Cambridge, USA

Till Photonics GmbH, München

WERNER BioAgents



# Internal Product Line

# Internal Product Line

For the identification of novel natural products from microbial and plant sources, the HKI follows a multidisciplinary approach based on intense cooperation and exchange between the different departments and junior research groups.
The activities within the HKI, and with external collaborators, are directed and coordinated by the IPL team. It consists of a group of scientists representing the different expertises required for the discovery, identification and characterization of natural products. The IPL-team establishes, maintains and improves the basic principles and platform technologies. These includes strain collection, compound libraries, assay methods and data management.

The departments and research groups provide microbial strains, extracts, natural products and synthetic derivatives. They have identified new targets, developed and run novel assay systems. Elucidation and optimization of biosynthetic pathways, strain improvement, pilot scale fermentation and downstream processing are carried out for natural products of primary interest. New technologies, like transcription profile analysis, proteome analysis and metabolomics, complement the methods applied.
Identified lead compounds are characterized in their function as mediators of biological communication. The IPL team evaluates the natural products as tools for modern biotechnology or as therapeutic agents.

## INTRODUCTION | EINLEITUNG

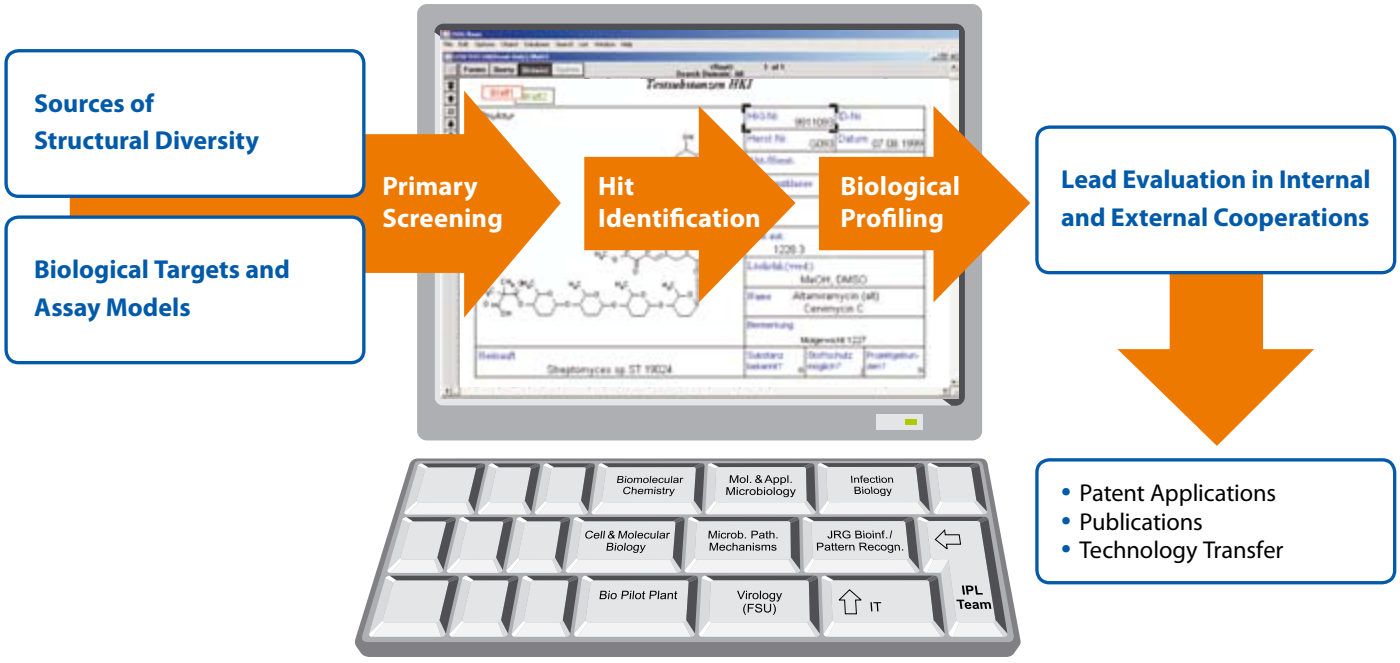
**Coordinator:**
**Dr. Ute Möllmann**

Zur Gewinnung neuer Naturstoffe aus Mikroorganismen und Pflanzen verfolgt das HKI einen multidisziplinären Ansatz. Er basiert auf einer intensiven Kooperation aller Abteilungen und Nachwuchsgruppen des Institutes, die zu diesem Zweck in der „Durchgehenden Bearbeitungslinie“ (DBL) zusammengeschlossen sind.
Alle damit verbundenen Aktivitäten innerhalb des HKI und mit externen Partnern werden vom DBL-Team koordiniert. Es setzt sich aus Fachleuten aller Arbeitsgruppen zusammen, die mit ihrer individuellen Expertise in alle Stufen der Bearbeitung therapeutisch interessanter neuer Naturstoffe einbezogen sind. Das DBL-Team entwickelt, etabliert und pflegt die methodischen und organisatorischen Grundlagen und Technologien einer professionellen vorklinischen Bearbeitung neuer Strukturen, wie zum Beispiel Stammsammlung, Substanzbibliothek, Datenbanken, biologische Testmodelle und Verfahren. Weiterhin steuert das DBL-Team Screeninghierarchien, Substanzflüsse und das Datenmanagement sowie die interdisziplinäre Auswertung und biologische Profilierung erfolgversprechender Hits. Durch langjährige direkte Kontakte zu Industriepartnern und eine enge Zusammenarbeit mit der Verwertungsagentur Ascenion werden bestehende und neu angemeldete Schutzrechte erfolgreich vermarktet.
Die Abteilungen und Forschungsgruppen tragen je nach ihrer Ausrichtung Mikroorganismenstämme, Extrakte, Naturstoffe und/oder synthetische Derivate zur DBL bei. Andere Gruppen identifizieren neue Targets, entwickeln Testsysteme und nutzen diese für die

The assays established and available for identification and characterization of natural products include a diversity of biological communication systems. They facilitate studies of interactions between natural products and biological macromolecules. This implies molecular interactions of natural products with microorganisms but also with higher organisms and interference with complex pathogen/host-interactions.
The IPL work focuses on new (antifungal) lead compounds, active against difficult to treat/ problematic fungal pathogens in humans.

Suche nach neuen Leitstrukturen. Für Naturstoffe von vorrangigem Interesse werden Biosynthesewege untersucht, die Produktbildner optimiert, sie werden im Technikumsmaßstab kultviert und aufgearbeitet. Neue Technologien wie die Transkriptom-, Proteom- und Metabolomanalyse vervollständigen das verfügbare Methodenspektrum.
Das DBL-Team evaluiert Naturstoffe hinsichtlich ihrer Nutzung als Werkzeuge für die moderne Biotechnologie oder als potentielle neue Therapeutika. In Projekten des HKI-eigenen Netzwerkes Grundlagenforschung werden neue Leitstrukturen in ihrer Funktion als Mediatoren der biologischen Kommunikation untersucht.
Die für die Identifizierung und Charakterisierung von Naturstoffen entwickelten Testsysteme umfassen eine große Vielfalt biologischer Kommunikationssysteme. Sie ermöglichen das Studium der Interaktion von Naturstoffen mit biologischen Makromolekülen und ihrer Wirkung innerhalb biologischer Netzwerke. Dies beinhaltet im wesentlichen molekulare Interaktionen mit Mikroorganismen, aber auch mit höheren Organismen einschließlich komplexer Pathogen-Wirt-Beziehungen. Ein Schwerpunkt der DBL-Arbeit besteht im Auffinden und der Bearbeitung neuer antifungaler Leitstrukturen, die gegen bislang nur schwer bekämpfbare Pilzerkrankungen des Menschen eingesetzt werden könnten.

# Internal Product Line (IPL)
## Durchgehende Bearbeitungslinie (DBL)




Departments and their IPL-team representatives

| | |
|---|---|
| IPL-Coordinator | Ute Möllmann |
| Biomolecular Chemistry | Peter Gebhardt<br>Corinna Lange<br>Isabel Sattler |
| Cell and Molecular Biology | Frank Hänel |
| Infection Biology | Hans-Martin Dahse |
| Microbial Pathogenicity Mechanisms | Ilse Jacobsen |
| Molecular and Applied Microbiology | Ute Möllmann<br>Barbara Schütze<br>Uta Wohlfeld |
| Administration | Michael Ramm (Deputy Coordinator)<br>Reinald Schorcht |
| Institute of Virology and Antiviral Therapy, University Hospital Jena | Michaela Schmidtke |

## HKI culture collection

The collection comprises more than 34.000 strains of actinomycetes, bacteria, fungi, yeasts and bacteriophages.

| | Genera | Species | Strains |
|---|---|---|---|
| Actinomycetes | 100 | 990 | 29.630 |
| Bacteria (without Actinomycetes) | 75 | 150 | 670 |
| Fungi and Yeasts | 428 | 590 | 3.594 |
| Bacteriophages | 22 | | 225 |

## HKI compound libraries

The HKI IPL compound basis consists of ca. 4.500 compounds. Additionally, the HKI maintains a natural products pool of approx. 9.000 compounds.

## HKI assays

For a primary characterization of compounds antibacterial, antifungal, antiviral, antiinflammatoric, antiproliferative and cytotoxicity assays are available. These include as test models a broad panel of Gram-positive and Gram-negative bacteria, yeasts, fungi, coxsackie virus B3 (CVB3), influenza virus A and *Herpes simplex* virus typ 1 (HSV1), L-929, K-562 and HeLa cell lines, assays for inhibitors of the oxidative burst in macrophages, inhibitors of 3α-hydroxy-steroid dehydrogenase (3α-HSD), xanthinoxidase and peroxidase. Yeast two-hybrid assays are applied for the identification of inhibitors of the protein-protein interaction between the oncogenic transcription factor Myc and the interacting protein Max, between the oncogenic transcription factor Myc and the interacting zinc-finger protein-Miz 1, as well as between the oncogenic retroviral Tax and CREB proteins. For secondary characterization of compounds additional *in vitro* assays are available, as well as *in vivo* models in the embryonated hens egg and in mice.

## HKI database

A multi-tiers database was established on ORAC-LE(TM). It joins the databases and the accumulated knowledge about microbial strains, their natural products and synthetic derivatives. The access to the broad structural diversity basis offered by the database supports the collaboration between HKI departments.

## Selected projects

**Cervimycin K** is produced as a minor component by a *Streptomyces tendae* strain isolated from a cave with prehistoric rock paintings in Italy. The compound is active against problematic pathogens like multiresistant staphylococci and vancomycin resistant enterococci. The potentially new target and the mechanism of action are under investigation.

**MEND** – The collaborative NIH funded project "Novel Derivatization and Functionalization of Natural Products", PI: Prof. Marvin J. Miller, University of Notre Dame, USA; (PI in the HKI: Ute Möllmann) focuses on "**M**odular **E**nhancement of **N**ature's **D**iversity (MEND)". The project involves members of the IPL Team and uses the broad spectrum of natural products available in the HKI compound library. Stabilized iminonitroso Diels-Alder reactions, particularly the pyridine nitroso Diels-Alder (NDA) reaction, is used as a remarkably efficient method for derivatization and functionalization of complex diene-containing natural products. Turimycin H3, ergosterol, reductiomycin, isoforocidin, colchicine and thebaine were found to react with nitrosopyridines in a highly efficient regio- and stereo-selective fashion. In most cases, NDA cycloadducts were obtained in more than 90% purity without any work-up or purification. Bioactivity of the compounds is studied by the HKI assays and screening hirarchie. Preliminary evaluations of the cycloadducts suggested that the nitroso heterocycles changed the biological activity profile of its parent natural product. The results of the project are published in several scientific journals.

**Benzothiazinones** – In our effort to discover new leads to counter the tuberculosis (TB) pandemic we synthesized and characterized 1,3-benzothiazin-4-ones (BTZs). BTZs are a new class of antimycobacterial agents that kill *Mycobacterium tuberculosis in vitro*, *ex vivo*, and in mouse models of TB. Within the EU-funded project "New medicines for tuberculosis" (NM4TB) the enzyme decaprenylphosphoryl-β-D-ribose 2'-epimerase was identified as a major BTZ target by cooperation partners. Inhibition


Li F, Yang B, Miller MJ, Zajicek J, Noll BC, Möllmann U, Dahse HM, Miller PA (2007) Iminonitroso Diels-Alder reactions for efficient derivatization and functionalization of complex diene-containing natural products. *Org Lett* 9, 2923-2926.

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Krchňák V, Moellmann U, Dahse H-M, Miller MJ (2008) Solid-Supported Nitroso Hetero Diels–Alder Reactions. 1. Acylnitroso Dienophiles: Scope and Limitations. *J Comb Chem* 10, 94–103

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Krchňák V, Moellmann U, Dahse H-M, Miller MJ (2008) Solid-Supported Nitroso Hetero-Diels–Alder Reactions. 3. Acid-Mediated Transformation of Cycloadducts by Scission of the Oxazine C–O Bonds. *J Comb Chem* 10, 112–117

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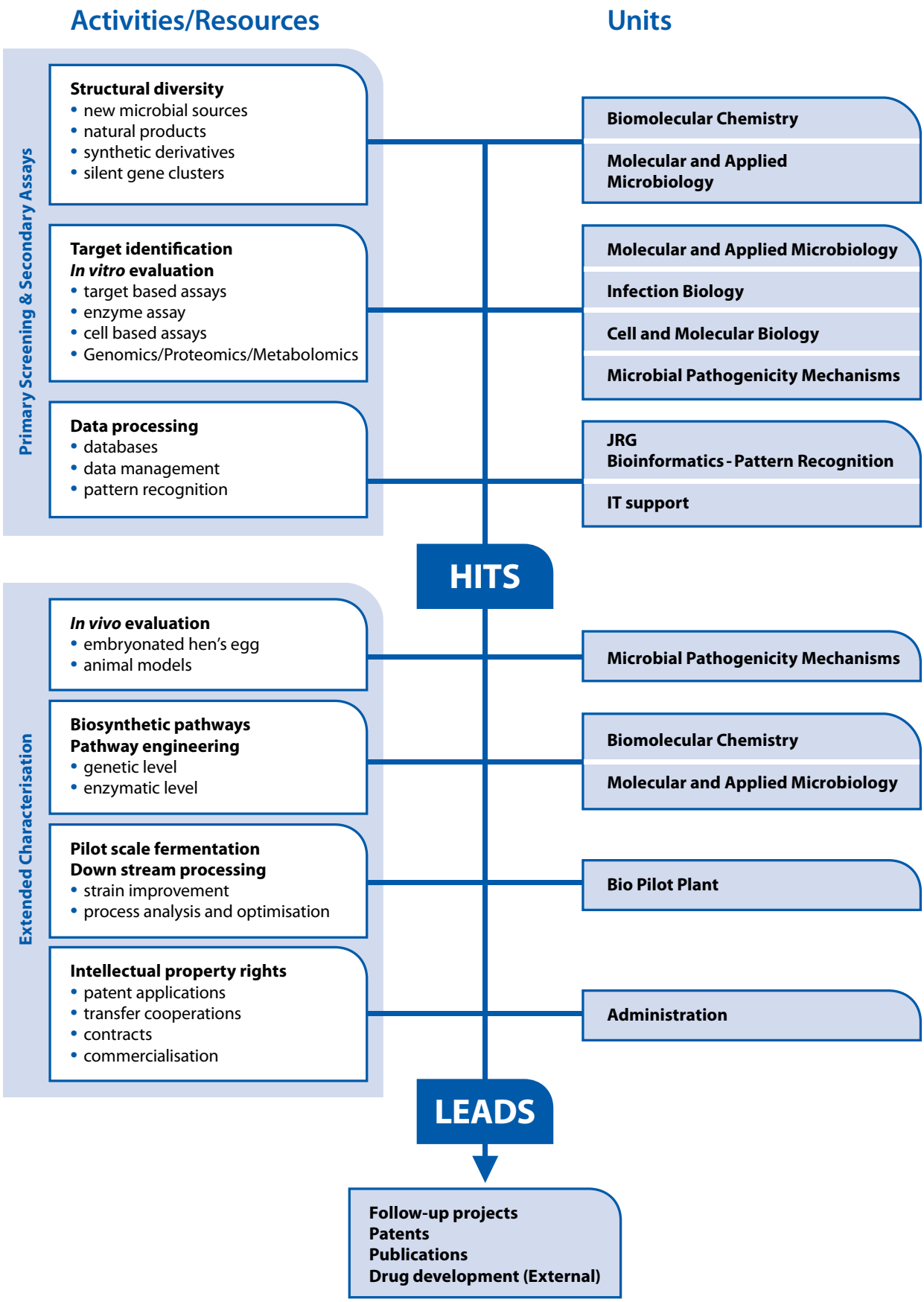
Makarov V, Manina G, Mikusova K, Möllmann U, Ryabova O, Saint-Joanis B, Dhar N, Pasca MR, Buroni S, Lucarelli AP, Milano A, De Rossi E, Belanova M, Bobovska A, Dianiskova P, Kordulakova J, Sala C, Fullam E, Schneider P, McKinney JD, Brodin P, Christophe T, Waddell S, Butcher P, Albrethsen J, Rosenkrands I, Brosch R, Nandi V, Bharath S, Gaonkar S, Shandil RK, Balasubramanian V, Balganesh T, Tyagi S, Grosset J, Riccardi G, Cole ST. Benzothiazinones kill *Mycobacterium tuberculosis* by blocking arabinan synthesis. *Science*, in press

Makarov VA, Cole S, Möllmann U. New thiazinone derivatives, their preparations and their use as antibacterials. PCT/EP2006/004942

Möllmann U, Makarov VA, Cole S. New antimicrobial compounds, their synthesis and their use for treatment of mammalian infection. EP2020406, PCT/EP2008/005142

of this enzymatic activity abolishes the formation of decaprenylphosphoryl arabinose, a key precursor that is required for the synthesis of the cell-wall arabinans, thus provoking cell lysis and bacterial death. BTZs were identified using a combination of activity-guided chemical and biotechnological techniques followed by extensive biological profiling within the IPL. The most advanced compound, BTZ043, is a candidate for inclusion in combination therapies for both drug-sensitive and extensively drug-resistant TB.

# Internal Product Line (IPL)
## Durchgehende Bearbeitungslinie (DBL)




# International Leibniz Research School

# International Leibniz Research School

The „International Leibniz Research School for Microbial and Biomolecular Interactions Jena“ (ILRS) is the first graduate school at a institute of the Leibniz Association. It is financed by competitively granted means of the Joint Initiative for Research and Innovation. After its foundation in autumn 2005, in March 2006 the first PhD students commenced their work at scientific projects. Beside the Leibniz Institute for Natural Product Research and Infection Biology – Hans Knöll Institute (HKI) – the Friedrich Schiller University of Jena (FSU) as well as the Max Planck Institute for Chemical Ecology (MPICE) are involved.
With the ILRS, the Hans Knöll Institute made a significant contribution to the profile of the Jena site as a centre of microbiological research in Germany and Europe. Research activities in the field of microbiology existing in Jena will be brought together and will be focussed on the aspect of interaction and communication of microorganisms. By doing this, an environment for excellent scientific performance will be created. More than half of the PhD students of the graduate school come from abroad. This enhances international networking and increases lastingly Jena's international attractiveness.

Die „International Leibniz Research School for Microbial and Biomolecular Interactions Jena“ (ILRS) ist die erste Graduiertenschule an einem Institut der Leibniz-Gemeinschaft. Sie wird aus kompetitiv vergebenen Mitteln des Paktes für Forschung und Innovation finanziert. Nach ihrer Gründung im Herbst 2005 begannen im März 2006 die ersten Doktoranden mit der Arbeit an ihren wissenschaftlichen Projekten. Neben dem Leibniz-Institut für Naturstoff-Forschung und Infektionsbiologie – Hans-Knöll-Institut (HKI) – sind die Friedrich-Schiller-Universität Jena (FSU) und das Max-Planck-Institut für chemische Ökologie (MPICE) beteiligt.
Mit der ILRS hat das Hans-Knöll-Institut einen wesentlichen Beitrag zur Profilierung des Standortes Jena als Zentrum der mikrobiologischen Forschung in Deutschland und Europa geschaffen. Die in Jena vorhandenen Forschungsaktivitäten auf dem Gebiet der Mikrobiologie werden gebündelt und auf den Aspekt Wechselwirkung und Kommunikation von Mikroorganismen fokussiert. Hierdurch werden die Voraussetzungen für herausragende wissenschaftliche Leistungen geschaffen. Mehr als die Hälfte der Doktoranden der Graduiertenschule kommt aus dem Ausland. Dies fördert die internationale Vernetzung und erhöht nachhaltig die internationale Attraktivität Jenas.
Im Rahmen der strukturierten Doktorandenausbildung nehmen die jungen Wissenschaftler an methodenspezifischen Praktika teil, veranstalten eigene Symposien- und Kolloquienreihen und pflegen den wissenschaftlichen Kontakt zu international renommierten Forschern

| PhD student | Supervisor |
|---|---|
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| Katrin Brzonkalik | Prof. Johannes Wöstemeyer |
| Alexander Funk | Dr. Uwe Horn |
| Christian Hummert | Dr. Reinhard Guthke,<br>Dr. Ulrich Möller |
| Radhika Jain | Prof. Axel A. Brakhage |
| Susann Jezewski | Prof. Erika Kothe |
| Gerald Lackner | Prof. Christian Hertweck |
| Hoang Hoa Long | Prof. Ian Baldwin |
| Shanshan Luo | Prof. Peter F. Zipfel |
| François Mayer | Prof. Bernhard Hube |

| Institute | Project title |
|---|---|
| Max Planck Institute for Chemical Ecology<br>Department of Bioorganic Chemistry | Inter- and intraspecies communication between soil bacteria – Induction of secondary metabolism |
| Friedrich Schiller University of Jena<br>Institute of General Microbiology and Microbial Genetics | Regulation of the sexual communication system in *zygomycetes* |
| Leibniz Institute for Natural Product Research and Infection Biology – Hans Knöll Institute<br>Bio Pilot Plant | Regulation of polyketide synthase gene clusters in *A. nidulans* |
| Leibniz Institute for Natural Product Research and Infection Biology – Hans Knöll Institute<br>Department of Molecular and Applied Microbiology<br>Systems Biology/Bioinformatics | Error correction for the integration of proteome and transcriptome data |
| Leibniz Institute for Natural Product Research and Infection Biology – Hans Knöll Institute<br>Department of Molecular and Applied Microbiology | Activation of MAP kinase networks of *Aspergillus fumigatus* during the interaction of the fungus with the host |
| Friedrich Schiller University of Jena<br>Institute of Microbiology - Microbial Phytopathology | Analysis of the pheromone/receptor system of *Schizophyllum commune* |
| Leibniz Institute for Natural Product Research and Infection Biology – Hans Knöll Institute<br>Department of Biomolecular Chemistry | Genomic studies of the endofungal, rhizoxin producing bacterium *Burkholderia rhizoxinica*, an endosymbiont of the zygomycete *Rhizopus microsporus* |
| Max Planck Institute for Chemical Ecology<br>Department of Molecular Ecology | Characterizing and analyzing the ecological consequences of the plant - endophyte interactions of *Solanum nigrum* and *Nicotiana attenuata* |
| Leibniz Institute for Natural Product Research and Infection Biology – Hans Knöll Institute<br>Department of Infection Biology | Immune evasion of pathogenic fungi |
| Leibniz Institute for Natural Product Research and Infection Biology – Hans Knöll Institute<br>Department of Microbial Pathogenicity Mechanisms | Identification and characterisation of infection-associated genes in *Candida albicans* |

In the framework of the structured PhD training the young scientists participate in method-specific internships and organize their own symposium and colloquium series and foster scientific contact with internationally renowned researchers in their field. In addition to the organization of such technical aspects of the training, the graduate school's coordinator is responsible for comprehensive socio-cultural care for the students, which has positive effects on a community forming. Included in the performance catalogue of the ILRS Jena is also a publicity related circulation of research results. All training-relevant measures will be completed by the PhD students according to a *Programme of Study* developed for the ILRS. This ensures highly efficient qualification of scientific junior staff within a very strict time schedule.

Scientific topics of the ILRS are interactions of microorganisms among each other as well as with higher developed organisms (e. g. as pathogens), natural products involved in this interaction as well as analysis and evaluation of the experimentally gained data by using methods of bioinformatics. Microorganisms do not occur in nature as single cells, but they live in very different habitats in association with other organisms. This includes mutual symbiosis as well as parasitic interactions. Basis for all such communication processes are substances that are synthesized by the organisms involved and are mutually sensed. A better understanding of the so far unexplored paths of communication will therefore help to trace new biologically active substances which have been optimized in terms of their effects by natural means in the course of evolution.

| PhD student | Supervisor |
|---|---|
| Jennifer Sneed | Prof. Georg Pohnert |
| Krisztina Truta-Feles | Prof. Johannes Norgauer |
| Katrin Volling | Prof. Hans Peter Saluz |
| YongQiang Wang | Prof. Günter Theißen |
| Lidan Ye | Prof. Gabriele Diekert |
| Hangxing Yu | Prof. Eberhard Straube |

ihres Fachgebietes. Neben der Organisation dieser fachlichen Seite der Ausbildung ist die Koordinatorin der Graduiertenschule für eine umfassende soziokulturelle Betreuung der Doktoranden verantwortlich, die sich positiv auf die Gemeinschaftsbildung auswirkt. Zum Leistungskatalog der ILRS Jena gehört auch die öffentlichkeitswirksame Verbreitung der Forschungsergebnisse. Alle ausbildungsrelevanten Maßnahmen werden von den Doktoranden entsprechend einem für die ILRS entwickelten *Program of Study* absolviert. Dies sichert eine hocheffiziente Qualifikation des wissenschaftlichen Nachwuchses in einem sehr stringenten Zeitschema.

Wissenschaftliche Themen der ILRS sind die Interaktionen von Mikroorganismen untereinander sowie mit höheren Organismen (z. B. als Pathogene), deren stoffliche Grundlage sowie die Analyse und Bewertung der experimentell gewonnenen Daten mit den Methoden der Bioinformatik. Mikroorganismen kommen in der Natur nicht als Einzelzellen vor, sondern leben in den verschiedensten Habitaten im Verbund mit anderen Organismen. Hierzu gehören mutualistische Symbiosen ebenso wie parasitäre Interaktionen. Grundlage sämtlicher Kommunikationsprozesse sind Substanzen, die von den beteiligten Organismen synthetisiert und wechselseitig sensiert werden. Ein besseres Verständnis der bisher weitgehend unerforschten Kommunikationswege dient damit der Auffindung neuer biologisch aktiver Substanzen, die im Laufe der Evolution auf natürlichem Wege in ihrer Wirkung optimiert wurden.

**Assoziierte Mitglieder**

| | |
|---|---|
| Daniela Albrecht | Dr. Reinhard Guthke,<br>Dr. Uwe Möller |
| Sandra Koers | Prof. Johannes Wöstemeyer |
| Stefanie Seitz | Prof. Maria Mittag |
| Felicitas Schöbel | Prof. Axel A. Brakhage,<br>Dr. Matthias Brock |

| Institute | Project title |
|---|---|
| Friedrich Schiller University of Jena<br>Institute for Inorganic and Analytical Chemistry<br>Bioorganic Analytics | Chemically mediated interactions between *Dictyosphaeria ocellata* and its associated microbial community |
| University Hospital Jena<br>Clinic for Dermatology | Function of phosphatidylinositol-3-kinase-γ and SH2-containing inositol-5-phosphatase-1 in innate immunity |
| Leibniz Institute for Natural Product Research and Infection Biology – Hans Knöll Institute<br>Department of Cell and Molecular Biology | Influence of *Aspergillus fumigatus* on apoptosis of host immune effector cells |
| Friedrich Schiller University of Jena<br>Department of Genetics | Reconstructing the interaction network of reproductive homeotic proteins in a gymnosperm |
| Friedrich Schiller University of Jena<br>Institute for Microbiology - Lehrstuhl für Angewandte und Oekologische Mikrobiologie | Bacterial communities from soil ecosystems possibly involved in lignin degradation |
| University Hospital Jena<br>Institute for Medical Microbiology | Modulation of host cell activation and apoptosis by *Chlamydia trachomatis* |
| Leibniz Institute for Natural Product Research and Infection Biology – Hans Knöll Institute<br>Department of Molecular and Applied Microbiology<br>Systems Biology/Bioinformatics | Integration of transcriptome and proteome data of human-pathogenic fungi |
| Max Planck Institute for Chemical Ecology<br>Department of Molecular Ecology | Proteome analysis on the development of the zygomycete *Mucor mucedo* |
| Friedrich Schiller University of Jena<br>Institute of General Botany | Functional characterization of molecular components of the circadian clock of the green alga *Chlamydomonas reinhardtii* |
| Leibniz Institute for Natural Product Research and Infection Biology – Hans Knöll Institute<br>Junior Research Group Microbial Biochemistry and Physiology | Impact of lysine biosynthesis of *Aspergillus* species in invasive aspergillosis |

# Network Fundamental Research

# Network Fundamental Research
# Netzwerk Grundlagenforschung

For the HKI, as member of the Leibniz Association, intensive networking between all departments, cross-sectional units, and junior research groups, as well as with external partners is one of the key features. In 2003, the Fundamental Research Network was established to strengthen this positive development of interdisciplinary cooperation. After a competitive evaluation procedure attended by the scientific advisory board, those projects which enter into new research areas will be awarded. This gives the scientists the opportunity to publish first research results and to prepare proposals for external funding in fundamental research areas. PhD fellowships are supported for up to 18 months. The doctoral candidates are supervised by scientists from at least two different departments and/or junior research groups of the HKI. The cooperation with other regional research institutions is highly appreciated. A summary about the funded projects and cooperation partners is shown in the following table.

Ein wesentliches Charakteristikum des HKI als Leibniz-Institut ist die enge Vernetzung der Abteilungen, Nachwuchsgruppen und Querschnittseinrichtungen miteinander und mit externen Partnern. Um diese positive Tendenz der interdisziplinären Zusammenarbeit weiter zu stärken, wurde im Jahre 2003 das Netzwerk zur Stärkung der Grundlagenforschung etabliert. Nach einem kompetitiven Auswahlverfahren, an dem der wissenschaftliche Beirat des HKI maßgeblich beteiligt ist, werden von Institutsmitarbeitern eingereichte Projekte gefördert, die neue Forschungsrichtungen erschließen sollen. Somit wird die Möglichkeit geschaffen, erste Ergebnisse zu publizieren und die erfolgreiche Einwerbung von Drittmitteln im Bereich der Grundlagenforschung vorzubereiten. Über eine Laufzeit von bis zu 18 Monaten werden Promotionsarbeiten gefördert, die von mindestens zwei verschiedenen Abteilungen und/oder Nachwuchsgruppen des HKI betreut werden. Eine Beteiligung anderer Institutionen der Region wird dabei angestrebt. Die nachfolgende Übersicht stellt die bisher geförderten Projekte und die daran beteiligten Kooperationspartner vor.

| Project number | Project leader<br>Partners |
|---|---|
| 2502 | Jürgen Wendland<br>Hans Peter Saluz<br>Ute Möllmann |
| 2503 | Anja Kunert<br>Michael Ramm |
| 2506 | Christian Hertweck<br>Hans-Martin Dahse<br>Martin Roth<br>Ute Möllmann |
| 2508 | Peter Zipfel<br>Christian Hertweck<br>Kerstin Herold<br>Hans Peter Saluz |
| 2511 | Frank Hänel<br>Christine Skerka |
| 2512 | Peter Zipfel<br>Christian Hertweck |
| 2513 | Dirk Schwartz<br>Ernst Roemer |

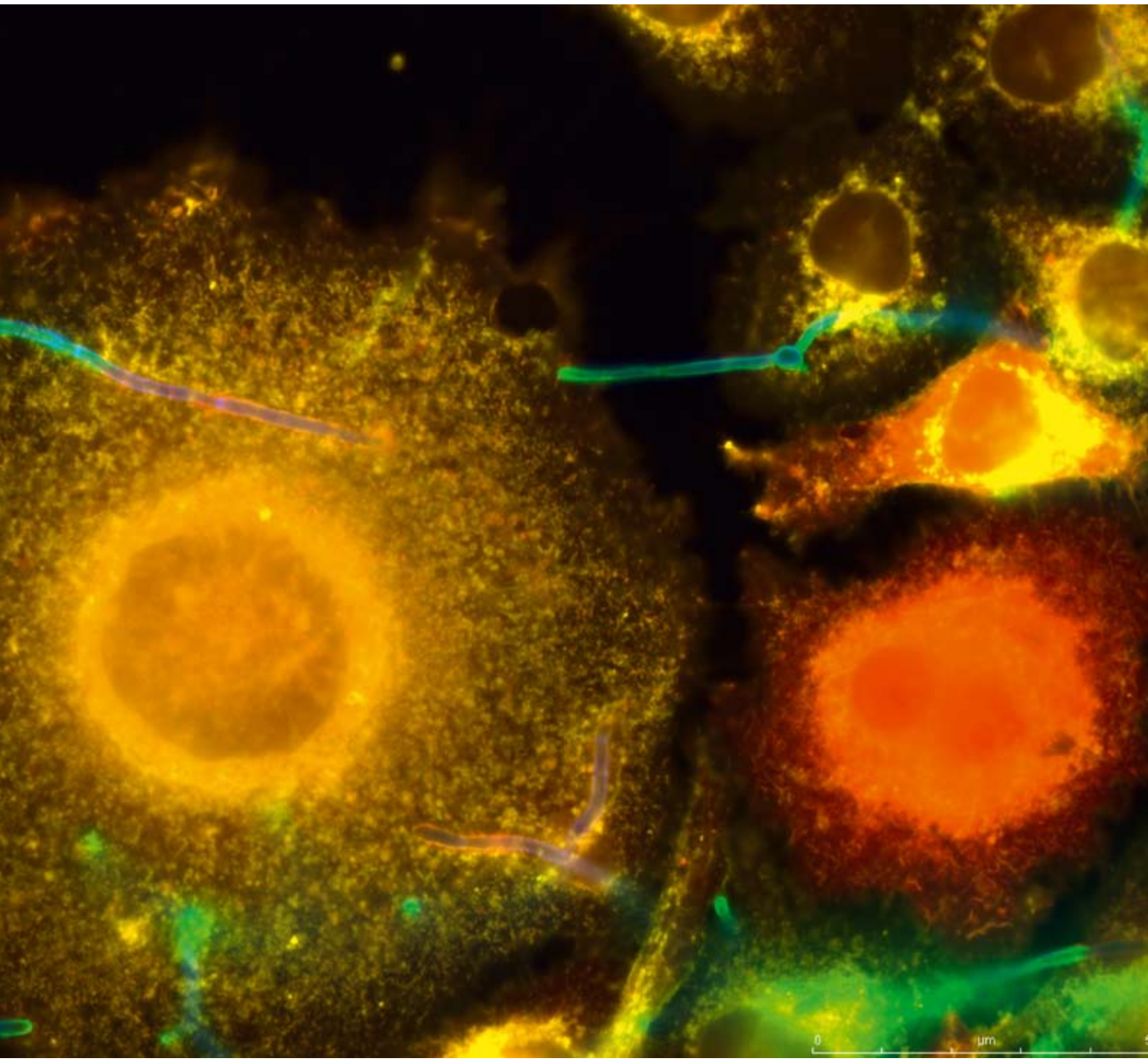
| | |
|---|---|
| 2601 | Christian Hertweck<br>Axel Brakhage<br>Peter Zipfel<br>Hans Peter Saluz |
| 2602 | Hans Peter Saluz<br>Axel Brakhage<br>Christian Hertweck<br>Peter Zipfel<br>Albert Härtl<br>Uwe Horn |
| 2603 | Axel Brakhage<br>Peter Zipfel<br>Albert Härtl |
| 2604 | Matthias Brock<br>Reinhard Guthke<br>Olaf Scheibner<br>Ulrich Möller |

| Unit | Project title | Project duration |
|---|---|---|
| Growth-Control of Fungal Pathogens<br>Cell and Molecular Biology<br>Infection Biology | Antifungale HKI-Naturstoffe und deren Wirkung auf genetische Pathways in *Candida albicans*: Ein neuartiger Weg zur Entschlüsselung potenter Targets | 12/2004 – 05/2006 |
| Infection Biology<br>Molecular and Applied Microbiology | Immunevasion von *Pseudomonas aeruginosa*: Charakterisierung der Faktor-H-Bindung | 11/2004 – 05/2006 |
| Biomolecular Chemistry<br>Infection Biology<br>Bio Pilot Plant<br>Infection Biology | Cervimycin-Projekt Teil A: Molekulare Grundlage der Cervimycin-Biosynthese – Funktionsanalyse und gerichtete Biosynthese von Cervimycin K und Analoga | 10/2004 – 03/2006 |
| Infection Biology<br>Biomolecular Chemistry<br>Biomolecular Chemistry<br>Cell and Molecular Biology | Cervimycin-Projekt Teil C: Wirkprofil und Wirktargets der Cervimycine mittels Proteomanalyse (Proteomics) | 10/2004 – 03/2006 |
| Cell and Molecular Biology<br>Infection Biology | Rolle der Zinkfinger-Transkriptionsfaktoren MIZ-1 und EGR-1 bei der Regulation des Zellwachstums und der Apoptose | 07/2004 – 02/2006 |
| Infection Biology<br>Biomolecular Chemistry | Einsatz eines Protein-Microarrays zur Wirkstoff-Testung | 12/2004 – 05/2006 |
| Molecular Antibiotics Biosynthesis<br>Molecular Natural Products Research | Untersuchungen zur Bereitstellung von aproteinogenen Aminosäuren in Antibiotikabiosynthesen | 10/2004 – 03/2006 |

| | | |
|---|---|---|
| Biomolecular Chemistry<br>Molecular and Applied Microbiology<br>Infection Biology<br>Cell and Molecular Biology | Targetanalyse und Wirkprofil antibakterieller Polyketidglykoside | 01/2006 – 07/2007 |
| Cell and Molecular Biology<br>Molecular and Applied Microbiology<br>Biomolecular Chemistry<br>Infection Biology<br>Microbial Pathogenicity Mechanisms<br>Bio Pilot Plant | Cervimycin: Aufnahme – Wirkung – Zytotoxizität | 01/2006 – 06/2007 |
| Molecular and Applied Microbiology<br>Infection Biology<br>Microbial Pathogenicity Mechanisms | Komplement-Evasion von *Aspergillus fumigatus* | 01/2006 – 06/2007 |
| Molecular and Applied Microbiology<br>Molecular and Applied Microbiology<br>Biomolecular Chemistry<br>Junior Research Group Bioinformatics – Pattern Recognition | Metabolomanalyse filamentöser Pilze zur Identifizierung virulenzdeterminierender Metabolite | 01/2006 – 06/2007 |

| Project number | Coordinator<br>Partners |
|---|---|
| 2605 | Christian Hertweck<br>Axel Brakhage<br>Jens Nielsen |
| 2606 | Olaf Kniemeyer<br>Martin Roth<br>Uwe Horn<br>Olaf Scheibner |
| 2607 | Axel Brakhage<br>Uwe Horn<br>Christian Hertweck<br>Albert Härtl |
| 2608 | Christian Hertweck<br>Christine Skerka<br>Hans-Martin Dahse<br>Peter Zipfel<br>Dirk Trauner |
| 2609 | Isabel Satttler<br>Ingrid Groth<br>Ute Möllmann<br>Uwe Horn |
| 2610 | Christine Skerka<br>Christian Hertweck |
| 2611 | Peter Zipfel<br>Jürgen Wendland |
| 2612 | Peter Zipfel<br>Peter Hortschansky |
| 2613 | Peter Zipfel<br>Bernhard Hube |
| 2614 | Christian Hertweck<br>Axel Brakhage |
| 2615 | Christian Hertweck<br>Martin Roth<br>Hans-Martin Dahse |
| 2618 | Hans Peter Saluz<br>Johannes Norgauer<br>Uta-Christina Hipler |
| 2626 | Christian Hertweck<br>Martin Roth<br>Jörn Piel |

| Unit | Project title | Project duration |
|---|---|---|
| Biomolecular Chemistry<br>Molecular and Applied Microbiology<br>Technical University of Denmark, Copenhagen | Analyse kryptischer Polyketidbiosynthese-Gencluster in *Aspergillus*: Cytochalasin-Synthase als Modellsystem | 01/2006 – 06/2007 |
| Molecular and Applied Microbiology<br>Bio Pilot Plant<br>Bio Pilot Plant<br>Biomolecular Chemistry | Proteomanalyse bei der Anpassung von *Aspergillus fumigatus* an hypoxische Bedingungen | 03/2006 – 08/2007 |
| Molecular and Applied Microbiology<br>Bio Pilot Plant<br>Biomolecular Chemistry<br>Microbial Pathogenicity Mechanisms | Generierung von DHN-Melanin Antikörpern zum Nachweis der Bedeutung von Melanin während der Infektion durch *Aspergillus fumigatus* | 01/2006 – 06/2007 |
| Biomolecular Chemistry<br>Infection Biology<br>Infection Biology<br>Infection Biology<br>University of California, Berkeley, USA | Chemo-Biosynthese von zytostatischen und immunosuppressiven Pyronverbindungen | 01/2006 – 06/2007 |
| Biomolecular Chemistry<br>Molecular and Applied Microbiology<br>Molecular and Applied Microbiology<br>Bio Pilot Plant | Sekundärmetabolite aus schwermetallresistenten seltenen Sporoactinomyceten aus dem Saalfelder Bergwerk „Feengrotten“ | 01/2006 – 06/2007 |
| Infection Biology<br>Biomolecular Chemistry | Entwicklung eines zellbasierenden Assays für die Identifizierung der Immunsuppression Wirkung von Naturstoffen | 01/2006 – 06/2007 |
| Infection Biology<br>Junior Research Group Growth-Control of Fungal Pathogens | Interaktion zwischen *Candida albicans* und menschlichen Immunzellen | 05/2006 – 10/2006 |
| Infection Biology<br>Molecular and Applied Microbiology | Bone Morphogenic Protein als Zielstrukturen für Mikroorganismen | 10/2006 – 03/2008 |
| Infection Biology<br>Microbial Pathogenicity Mechanisms | Degradation der extrazellulären Matrix des Wirtes durch die humanpathogene Hefe *Candida albicans* | 08/2007 – 02/2009 |
| Biomolecular Chemistry<br>Molecular and Applied Microbiology | Chinolin- und Benzodiazepin-Biosynthese in *Aspergillus nidulans* | 06/2007 – 12/2008 |
| Biomolecular Chemistry<br>Bio Pilot Plant<br>Infection Biology | Chemo-Biosynthese von antifungalen und antitumoralen Rhizoxin-Derivaten | 02/2007 – 07/2008 |
| Cell and Molecular Biology<br>University Hospital Jena<br>University Hospital Jena | Rapid molecular diagnostics of fungal infections of the human skin | 03/2007 – 08/2008 |
| Biomolecular Chemistry<br>Bio Pilot Plant<br>University Bonn | Genome Mining von anaeroben Naturstoffproduzenten | 12/2007 – 05/2009 |




# Appendix

# Appendix

**Peer Reviewed Articles 2006**
**Originalarbeiten 2006**

### Department Biomolecular Chemistry

Günther S, Groth I, **Grabley S**, Munder T (2006) Design and evaluation of an oligonucleotide-Microarray for the detection of diferrent species of the genus *Kitasatospora. J Microbiol Methods* 65, 226-236.

Huang X, Roemer E, **Sattler I**, Möllmann U, Christner A, Grabley S (2006) Lydiamycins A-D: cyclodepsipetides with antimycobacterial properties. *Angew Chem Int* Ed 45, 3067-3072.

**Ishida K**, Maksimenka K, **Fritzsche K**, **Scherlach K**, Bringmann G, **Hertweck C** (2006) The boat-shaped polyketide resistoflavin results from re-facial central hydroxylation of the discoid metabolite resistomycin. *J Am Chem Soc* 128, 14619-14624.

James TY, Kauff F, Schoch C, Matheny PB, Hofstetter V, Cox CJ, Celio G, Gueidan C, Fraker E, Miadlikowska J, Lumbsch HT, Rauhut A, Reeb V, Arnold AE, Amtoft A, Stajich JE, Hosaka K, Sung G-H, Johnson D, O'Rourke B, Crockett M, Binder M, Curtis JM, Slot JC, Wang Z, Wilson AW, Schüßler A, Longcore JE, O'Donnell K, Mozley-Standridge S, Porter D, Letcher PM, Powell MJ, Taylor JW, White MM, Griffith GW, Davies DR, Humber RA, Morton JB, Sugiyama J, Rossman AY, Rogers JD, Pfister DH, Hewitt D, Hansen K, Hambleton S, Shoemaker RA, Kohlmeyer J, Volkmann-Kohlmeyer B, Spotts RA, Serdani M, Crous PW, Hughes KW, Matsuura K, Langer E, Langer G, Untereiner WA, Lücking R, Büdel B, Geiser DM, Aptroot A, Diederich P, **Schmitt I**, Schultz M, Yahr R, Hibbett DS, Lutzoni F, McLaughlin DJ, Spatafora JW, Vilgalys R (2006) Reconstructing the early evolution of the fungi using a six gene phylogeny. *Nature* 443, 818-822.

Kniemeyer O, Lessing F, **Scheibner O, Hertweck C**, Brakhage AA (2006) Optimisation of a 2-D gel electrophoresis protocol for the human-pathogenic fungus *Aspergillus fumigatus*. *Curr Genet* 49, 178-189.

Krügel H, Becker A, Polten A, Grecksch G, Singh R, **Berg A**, Seidenbecher C, Saluz HP (2006) Transcriptional response to the neuroleptic-like compound ampullosporin A in the rat ketamine model. *J Neurochem* 97, 74-81.

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Li X, Zheng Y, **Sattler I**, Lin W (2006) Griseusin C, a novel quinone derivative from a marine-derived fungus *Penicillium sp. Arch Pharm Res* 29, 942-945.

**Müller M**, **He J**, **Hertweck C** (2006) Dissection of the late steps in aureothin biosynthesis. *ChemBioChem* 7, 37-39.

**Müller M**, **Kusebauch B**, Liang G, Beaudry CM, Trauner D, **Hertweck C** (2006) Photochemical origin of SNF4435C/D and formation of orinocin through 'polyene splicing'. Angew Chem 118, 7999-8002; *Angew Chem Int Ed* 45, 7835-7838.

Opitz A, Wei-Opitz D, **Gebhardt P**, Koch R (2006) An unusual reaction of the natural compound benaphthamycin B: theoretical study of a model system. *J Org Chem* 71, 1074-1079.

**Rassmann A**, Henke A, Zobawa M, Carlsohn M, Saluz HP, **Grabley S**, Lottspeich F, Munder T (2006) Proteome alterations in human host cells infected with coxsackievirus B3. *J Gen Virol* 87, 2631-2638.

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**Wangun HV**, Härtl A, Kiet TT, **Hertweck C** (2006) Inotilone and related phenylpropanoid polyketides from *Inonotus sp*. and their identification as potent COX and XO inhibitors. *Org Biomol Chem* 4, 2545-2548.

**Winkler R**, **Richter MEA**, Knüpfer U, Merten D, **Hertweck C** (2006) Regio- and chemoselective enzymatic N-oxygenation *in vivo*, *in vitro*, and *in flow*. *Angew Chem* 118, 8184-8186; *Angew Chem Int* Ed 45, 8016-8018.

### Department Cell and Molecular Biology

Ehricht R, Slickers P, **Goellner S**, Hotzel H, Sachse K (2006) Optimized DNA microarray assay allows detection and genotyping of single PCR-amplifiable target copies. *Mol Cell Probes* 20, 60-63.

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Groth I, Tan GY, Gonzalez JM, Laiz L, **Carlsohn MR**, Schütze B, Wink J, Goodfellow M (2007) *Amycolatopsis nigrescens sp. nov*., an actinomycete isolated from a Roman catacomb. Int *J Syst Evol Microbiol* 57, 513-519.
Günther S, Groth I, Grabley S, **Munder T** (2006) Design and evaluation of an oligonucleotide microarray for the detection of different species of the genus *Kitasatospora*. *J Microbiol Methods* 65, 226-236.

John N, **Krügel H**, Frischknecht R, Smalla KH, Schultz C, Kreutz MR, Gundelfinger ED, Seidenbecher C. (2006) Brevican-containing perineuronal nets of extracellular matrix in dissociated hippocampal primary cultures. *Mol Cell Neurosci* 31, 774-784.

**Krügel H**, Becker A, Polten A, Grecksch G, **Singh R**, Berg A, Seidenbecher C, **Saluz HP** (2006) Transcriptional response to the neuroleptic-like compound Ampullosporin A in the rat ketamine model. *J Neurochem* 97, 74-81.

**Nestler M**, Martin U, Hortschansky P, **Saluz HP**, Henke A, **Munder T** (2006) The zinc containing pro-apoptotic protein Siva interacts with the peroxisomal membrane protein pmp22. *Mol Cell Biochem* 287, 147-155.

Rassmann A, Henke A, Zobowa M, **Carlsohn M**, **Saluz HP**, Grabley S, Lottspeich F, **Munder T** (2006) Proteome alterations in human host cells infected with coxsackievirus B3. *J Gen Virol* 87, 2631-2638.

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**Usskilat C**, Skerka C, **Saluz HP**, **Hänel F** (2006) The transcription factor Egr-1 is a regulator of the human TopBP1 gene. *Gene* 8, 144-150.

Wendland J, Hellwig D, Walther A, Sickinger S, Shadkchan Y, Martin R, Bauer J, Osherov N, **Tretiakov A**, **Saluz HP** (2006) Use of the Porcine Intestinal Epithelium (PIE)-Assay to analyze early stages of colonization by the human fungal pathogen *Candida albicans. J Basic Microbiol* 46, 513-523.

**Department Infection Biology**


Abrera-Abeleda MA, Nishimura C, Smith JL, Sethi S, McRae JL, Murphy BF, Silvestri G, **Skerka C**, Jozsi M, **Zipfel PF**, Hageman GS, Smith RJ (2006) Variations in the complement regulatory genes factor H (CFH) and factor H related 5 (CFHR5) are associated with membranoproliferative glomerulonephritis type II (dense deposit disease). *J Med Genet* 43, 582-589.

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Cordes FS, Kraiczy P, Roversi P, Simon MM, Brade V, Jahraus O, Wallis R, Goodstadt L, Ponting CP, **Skerka C**, **Zipfel PF**, Wallich R, Lea SM (2006) Structure-function mapping of BbCRASP-1, a complement factor H-binding-virulence factor from *Borrelia burgdorferi*. *Int J Med Microbio*l 296,177-184.

Franke K, **Nguyen M**, Härtl A, **Dahse H-M**, Vogl G, Würzner R, **Zipfel PF**, Künkel W, **Eck R** (2006) The vesicle transport protein Vac1p is required for virulence of *Candida albicans*. *Microbiology* 152, 3111-3121.

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Gottschaldt M, Pfeifer A, Görls H, Obata M, **Dahse H-M**, **Möllmann U**, Yano S (2006) Unprecedented sugar depending silver(I) complexes with antimicrobial activity. *Tetrahedron*, 1-8.

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### Department Infection Biology

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### Junior Research Group Growth-Control of Fungal Pathogens

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### Junior Research Group Microbial Biochemistry and Physiology

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## Memberships in Editorial Boards 2006/2007
## Mitgliedschaften in Editorial Boards 2006/2007

**Brakhage, Axel**
Applied and Enviromental Microbiology, American Society for Microbiology, Washington DC
Current Genetics, Springer, Berlin
Archives of Microbiology, Springer, Berlin
Applied Microbiology and Biotechnology, Springer, Berlin
Microbiological Research, Elsevier, Jena

**Saluz, Hans Peter**
BioMethods, Springer, Berlin
Reviews in Molecular Biotechnology, Elsevier, Heidelberg

**Zipfel, Peter F.**
Molecular Immunology
Pediatric Nephrology
Thrombosis and Hemostasis

**Hube, Bernhard**
BMC Microbiology
Journal of Medical Microbiology
FEMS Yeast Research
**Horn, Uwe**
Microbiological Research

## Lectures at the HKI 2006
## Kolloquien am HKI 2006

Selbig, Joachim (MPI Molekulare Pflanzenphysiologie, Potsdam-Golm)
Integrated Analysis of Metabolite, Gene expression and other profile Data
31.01.2006
Host: Guthke R

Schreiber, Joerg
Coordinated action of NF-kappaB family members in the response of human cells to lipopolysaccaride
09.02.2006
Host: Zipfel PF

Hanley, Peter J.
Kockout mice reveal the contributions of P2Y and P2X receptors to nucleotide-induced Ca2+ signaling in macrophages
09.02.2006
Host: Zipfel PF

Józsi, Mihály (HKI, Jena)
Innate immunity in self defense
09.02.2006
Host: Zipfel PF

Arlt, Michael (Merck KgaA, Global Medicinal Chemistry Lead Finding)
Mangelnde Qualität von Screening-Verbindungen kann im Hit to Lead Prozess Probleme verursachen
08.03.2006
Host: Hertweck C

Kothe, Erika (Friedrich-Schiller-Universität Jena, Inst. für Mikrobiologie)
Schwermetallresistente Streptomyceten und ihre potentielle Nutzung in der Bioremediation
04.04.2006
Host: Roth M

Fotso, Serge (Universität Göttingen)
Bhimamycins and Other New Metabolites with Unusual Chromophores From Microorganisms
21.04.2006
Host: Sattler I

Elsner, Peter; Hipler, Uta-Christina (Universitätsklinikum Jena)
Aktuelle Probleme der dermatologischen Mykologie
25.04.2006
Host: Zipfel PF, Stelzner A, Brakhage AA

Groth, Ingrid (HKI, Jena)
Actinomyceten in den Saalfelder Feengrotten
25.04.2006
Host: Merten D - Uni, Jena

Feldbrügge, Michael (Max-Planck-Institut für Terrestrische Mikrobiologie, Marburg)
The role of RNA-binding proteins during pathogenic development of *Ustilago maydis*
09.05.2006
Host: Brakhage AA

Reinhart, K. (Universitätsklinikum Jena, Klinik für Anästhesiologie und Intensivmedizin)
Sepsis als medizinische und gesellschaftliche Herausforderung
16.05.2006
Host: Zipfel PF, Stelzner A, Brakhage AA

Böcker, Sebastian (Friedrich-Schiller-Universität Jena, Institut für Informatik, Lehrstuhl für Bioinformatik)
Algorithms for Interpreting Mass Spectrometry Data
23.05.2006
Host: Guthke R

Miller, Marvin J. (University of Notre Dame, Notre Dame, IN, USA)
New methods for the design and discovery of novel therapeutic Agents
30.05.2006
Host: Möllmann U, Zipfel PF

Li, Shu-Ming (Inst. für Pharm. Biologie und Biotechnologie, Heinrich-Heine-Universität Düsseldorf)
Biosynthesis of prenylated indole alkaloids in *Aspergillus fumigatus*
08.06.2006
Host: Brakhage AA

Glocker, Michael (Proteomzentrum der Universität Rostock)
Klinische Transkriptom- und Proteomanalyse: Interaktion von Wet-Lab und Dry-Lab
20.06.2006
Host: Guthke R

Preissner, Klaus T. (University Giessen)
Trick-or-treat with S. *aureus* Eap, a bacterial adhesion protein with therapeutic potential for vascular diseases
04.07.2006
Host: Zipfel PF

Bailly, Xavier
Recombination, specialisation and speciation of *Sinorhizobium* symbiotic bacteria that are associated to the genus *Medicago*
11.07.2006
Host: Hertweck C

Vogel, Ulrich (Inst. für Hygiene und Mikrobiologie, Würzburg)
Meningococcal disease and carriage: molecular epidemiology and experimental models
25.07.2006
Host: Zipfel PF

Goodship, Tim (Reader in Nephrology, Royal Victoria Infirmary, Newcastle upon Tyne)
Genomic disorders in the RCA cluster predispose to atypical HUS
14.08.2006
Host: Zipfel PF

Schwienhorst, Andreas (Georg-August-Universität Göttingen)
Histone deacetylases? Enzyme characterization and inhibitor design
22.08.2006
Host: Lange C

Mack, Matthias (Biotechnology Dept. Mannheim University of Applied Sciences)
Identification and characterization of two *Streptomyces davawensis* riboflavin biosynthesis gene clusters
10.10.2006
Host: Horn U

Wolf, G. (Klinik für Innere Medizin III, Friedrich-Schiller-Universität Jena)
Molekulare Mechanismen der diabetischen Nephropathie
17.10.2006
Host: Zipfel PF, Stelzner A, Brakhage AA

Roth, Martin (HKI, Jena)
Bad bugs, no drugs! How to fill the pipeline?
07.11.2006

Zintl, F. (Universitätskinderklinik Jena)
Stammzelltransplantation – Klinik, Erfolge, Probleme
21.11.2006
Host: Zipfel PF, Stelzner A, Brakhage AA

Vanderplasschen, Alain (Faculty of Veterinary Medicine, University of Liège, Belgium)
Inhibition of host complement by Ixodes tick salivary proteins
05.12.2006
Host: Zipfel PF

**Lectures at the HKI 2007**
**Kolloquien am HKI 2007**

Klipp, Edda (Max-Planck-Institut für Molekulare Genetik, Berlin, Kinetic Modeling Group)
Structural and dynamic properties of signaling pathways
16.01.2007
Host: Guthke R

van den Elsen, Jean
(University of Bath, England)
*Staphylococcus* aureus adhesion protein Sbi, a versatile inhibitor of the adaptive and innate immune systems?
23.01.2007
Host: Zipfel PF

Henke, Andreas (Institut für Virologie und antivirale Therapie, Universitätsklinikum Jena)
Viral heart diseases - balance between virus-caused pathology and immune response
13.03.2007
Host: Zipfel PF, Stelzner A, Brakhage AA

Pfister, Wolfgang (Institut für Medizinische Mikrobiologie, Friedrich-Schiller-Universität Jena)
Aktuelle Aspekte von Tropenkrankheiten
22.05.2007
Host: Zipfel PF, Stelzner A, Brakhage AA

Opfermann, Thomas (HKI, Jena)
Projekt: PET-CT Scanner am HKI, Grundlagen und Einführung
29.05.2007
Host: Saluz HP

Wells, Martin (Nonlinear Dynamics, Newcastle, UK)
Understanding and addressing the challenges of differential expression analysis of proteomic data
06.06.2007
Host: Kniemeyer O

Thewes, Sascha
(Robert-Koch-Institut, Berlin)
Liver invasion of *Candida albicans*
03.07.2007
Host: Hube B

Zocher, Rainer (TU Berlin, Biochemie und Molekularbiologie)
Biosynthesis of fungal N-methyl peptides
05.07.2007
Host: Brakhage AA

Hallström, Teresia
(University Lund, Schweden)
Survival strategies of the human respiratory pathogen *Haemophilus influenzae*
10.07.2007
Host: Zipfel PF

Mayer, Francois (Technical University of Braunschweig)
Proteome analysis of *Pseudomonas aeruginosa* biofilms under starvation conditions
19.07.2007
Host: Hube B

Moore, Bradley S. (Scripps Institution of Oceanography, UCSD, La Jolla, CA)
Applying genomics in the natural product drug discovery process
08.10.2007
Host: Hertweck C

Sickmann, Albert (Rudolf-Virchow Zentrum, DFG-Forschungszentrum für Experimentelle Medizin, Würzburg)
Membrane Proteomics
09.10.2007
Host: Brakhage AA, Kniemeyer O

Filler, Scott (Los Angeles Biomedical Research Institute, USA)
Als proteins and *C. albicans* invasion of host cells
10.10.2007
Host: Brakhage AA, Hube B

Odds, Frank
(University of Aberdeen, UK)
*Candida albicans*: phylogenetics, epidemiology and pathogenesis of Infection
11.10.2007
Host: Brakhage AA, Hube B

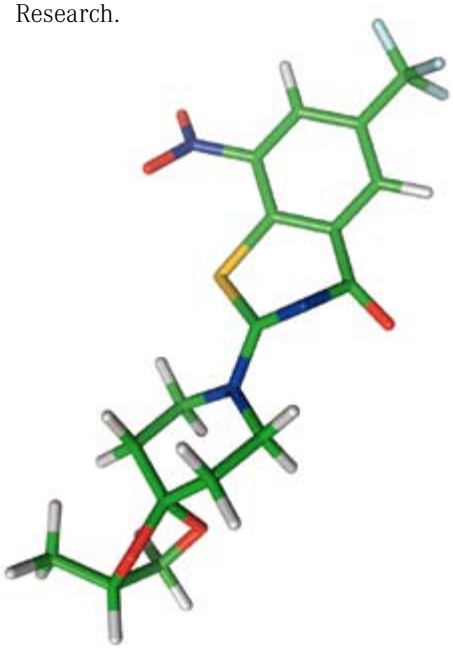
Bauer, Michael (Klinik für Anästhesiologie und Intensivtherapie, Universitätsklinikum Jena)
Molekularbiologie des septischen Organversagens
16.10.2007
Host: Zipfel PF, Stelzner A, Brakhage AA

Munro, Carol (University of Aberdeen, Institute of Medical Sciences, Aberdeen, UK)
*Candida albicans* cell wall integrity and salvage mechanisms
04.12.2007
Host: Hube B

Fleckenstein, Bernhard (Institut für Klinische und Molekulare Virologie, Universität Erlangen-Nürnberg)
Neue Viren
11.12.2007
Host: Zipfel PF, Stelzner A, Brakhage AA

## Inventions and Patents 2006/2007
## Erfindungen und Schutzrechte 2006/2007

Intellectual property rights as well as publications in peer reviewed journals are main performance parameters reflecting the quality of research at the HKI. In 2006/2007 a multitude of inventions were filed by departments focusing on natural product research and technology-oriented groups broadening the patent-portfolio of the institute. HKI patents resulted in a number of fruitful co-operations with industry and affected the institute's budget advantageously. HKI scientists developed together with two international cooperation partners a new drug against the causal agent of tuberculosis. The promising lead will be licensed to a pharmaceutical company for the further development of a novel anti-tuberculosis medicine. The application for new patents is stringently evaluated in the HKI and focuses on new biologically active natural products and their (bio-)synthetic derivatives. Since 2006 the HKI co-operates with Ascenion GmbH, Munich, to further optimize technology transfer and commercialization activities. This project is funded by the Federal Ministry of Education and Research.



2-[(2S)-2-methyl-1,4-dioxa-8-azaspiro[4.5]dec-8-yl]-8-nitro-6-(trifluoromethyl)-4H-1,3-benzothiazin-4-one (BTZ043) blocks growth of the tuberculosis pathogen *Mycobacterium tuberculosis* by inhibition of the enzymatic synthesis of cell wall associated arabinogalactan and lipoarabinomannan.

Schutzrechte sind neben Originalpublikationen in referierten Fachjournalen ein wesentlicher Leistungsparameter für die Forschungsarbeit am HKI. Sowohl die naturstoffchemisch arbeitenden Abteilungen als auch Technologieorientierte Gruppen trugen im Zeitraum 2006/2007 mit einer Vielzahl von Erfindungen zum Schutzrechts-Portfolio des Instituts bei. Vom HKI angemeldete Patente führten zu einer Reihe fruchtbarer Industriekooperationen und wirkten sich positiv auf das Budget des Instituts aus. In Kooperation mit einem Gastwissenschaftler aus Moskau und dem Institut Pasteur in Paris wurde am HKI ein neuer Wirkstoff gegen den Tuberkulose-Erreger entwickelt, der auch gegen resistente Stämme hochwirksam ist. Mit der Pharmaindustrie laufen Verhandlungen über eine Lizenzierung der Substanz zur Entwicklung eines neuen Medikamentes. Die Anmeldung neuer Schutzrechte unterliegt einer strengen hausinternen Evaluation und wird sich verstärkt auf neue, biologisch aktive Naturstoffe und deren (bio-)synthetische Derivate konzentrieren. In einem BMBF-geförderten Projekt zur effektiven Verwertung der Schutzrechte arbeitet das HKI seit 2006 erfolgreich mit der Ascenion GmbH, München zusammen.

**Scherlach K**, **Dahse H-M**, **Hertweck C**
Aspoquinolone, zytotoxische Verbindungen aus *Aspergillus nidulans*
DE 10 2006 006 893
priority establishing patent application: 08.02.2006

Makarov VA, Cole S, **Möllmann U**
New thiazinone derivatives, their preparations and their use as antibacterials
PCT/EP2006/004942
priority establishing patent application: 24.05.2006

Wutzler P, Makarov VA, Schmidtke M, **Dahse H-M**
4-Amino-3-arylamino-6-arylpyrazolo[3,4-d] pyrimidin-Derivate, Verfahren zu ihrer Herstellung und deren Verwendung als antivirale Wirkstoffe
DE 10 2006 029 074.7 • PCT/DE2007/001104
priority establishing patent application: 22.06.2006

**Möllmann U**, **Heinemann I**, Vogt S, Schnabelrauch M, Kautz A
Antibiotic maduraphthalazin salts with delayed release of the active substance and their use
EP 1908754
priority establishing patent application: 19.09.2006

**Müller PJ**, Ozegowski J-H, **Peschel G**, **Presselt R**, **Siering A**
New inhibitor of microbial hyaluronate lyase
EP 06025629.4 • PCT/EP2007/010902
priority establishing patent application: 12.12.2006

**Müller PJ**, **Krauter G**, **Presselt R**, Ozegowski J-H
Vorrichtung und Verfahren zur Durchführung von Emerswachstum bei Mikroorganismen und Zellen
DE 10 2007 005 744.1-41
priority establishing patent application: 31.01.2007

**Müller PJ**, Ozegowski J-H, **Roth M**, **Martin K**, Metze J, Riesenberg R, **Krauter G**
Vorrichtung und Verfahren zur Durchführung von emersen Mikrokultivierungen bei Mikroorganismen und Zellen
DE 10 2007 010 866.6-41
priority establishing patent application: 02.03.2007

**Horn U**, **Habicht G**, **Hortschansky P**, Fändrich M
Anti-amyloid antibodies and their use in diagnosis and therapy of amyloid diseases
EP 07090073.3 • PCT/EP2008/002826
priority establishing patent application: 05.04.2007

**Möllmann U**, Makarov VA, Cole S
New antimicrobial compounds, their synthesis and their use for treatment of mammalian infection
EP2020406 • PCT/EP2008/005142
priority establishing patent application: 16.07.2007

Lehmann J, Konter J, **Möllmann U**, **Härtl A**
Neue Ketoconazol-Derivate sowie deren Herstellung und Verwendung als antimikrobielle und antiinflammatorische Wirkstoffe
DE 102007035323.7
priority establishing patent application: 25.07.2007

Venbrocks RA, Kinne RW, Jandt KD, Bossert J, **Hortschansky P,** Schmuck K
Calcium phosphate based delivery of growth and differentiation factors to compromised bone
EP2033598
priority establishing patent application: 21.11.2007

## Scientific Awards 2006/2007
## Preise und Auszeichnungen 2006/2007

**Brakhage, Axel A.**
Heinz P.R. Seeliger-Preis für Medizinische Bakteriologie und Mykologie
2006

**Heinen, Stefan**
Hans Hench-Preis für Klinische Immunologie der Deutschen Gesellschaft für Immunologie 2006

**Metsä-Ketelä, Mikko**
EMBO-Stipendium
01.12.2005-31.03.2006

**Schenk, Angela**
Humboldt-Stipendium
01.02.2005-30.11.2006

**Walther, Andrea**
Nachwuchspreis der Leibniz-Gemeinschaft
2006

**Albrecht, Antje**
Wissenschaftspreis für eine herausragende wissenschaftliche Publikation auf dem Gebiet der medizinischen Mykologie auf der wissenschaftlichen Tagung der Deutschsprachigen Mykologischen Gesellschaft (DMykG), Berlin
2007

**Behnsen, Judith**
Wissenschaftspreis für eine herausragende wissenschaftliche Publikation auf dem Gebiet der medizinischen Mykologie auf der wissenschaftlichen Tagung der Deutschsprachigen Mykologischen Gesellschaft (DMykG), Berlin
2007

**Bergmann, Sebastian**
medac-Forschungspreis
2007

**Brock, Matthias**
medac-Forschungspreis
2007

**Hortschansky, Peter**
medac-Forschungspreis
2007

**Józsi, Mihály**
medac-Forschungspreis
2007

**Lange, Corinna**
medac-Forschungspreis
2007

**Scherlach, Kirstin**
medac-Forschungspreis
2007

**Schümann, Julia**
medac-Forschungspreis
2007

**Strobel, Stefanie**
medac-Forschungspreis
2007

**Wächtler, Betty**
Hans Rieth-Posterpreis für das beste Poster auf der 41. wissenschaftlichen Tagung der Deutschsprachigen Mykologischen Gesellschaft (DMykG), Berlin
2007

**Winkler, Robert**
medac-Forschungspreis
2007

**Zipfel, Peter**
Heinz-Spitzbart-Preis der Europäischen Gesellschaft für Infektionskrankheiten in der Geburtshilfe und Gynäkologie
2007

## Meetings, Workshops, Symposia 2006/2007
## Wissenschaftliche Veranstaltungen 2006/2007

1st ILRS Group Seminar
**Schmidt D, Brakhage AA**
Jena, Germany
2006

DFG-SPP 1160 Schwerpunkttagung "Colonisation and infection by human-pathogenic fungi"
**Brakhage AA**
Jena, Germany
2006

Jahrestagung der Vereinigung für Allgemeine und Angewandte Mikrobiologie (VAAM)
**Brakhage AA**, Diekert G, Kothe E, Straube E, Wöstemeyer J
Jena, Germany
2006

Meeting und Workshop „Schnelle Pathogendiagnostik mittels Rapid-PCR"
**Saluz HP**
Jakarta, Indonesien
2006

Practical course on "Rapid heatblock thermocycling of small samples in miniaturized ultrathin-walled microplates"
**Saluz HP**
Jena, Germany
2006

Programm-Seminar „Durchfluss Microfluidic RT-PCR"
**Krügel H**
Jena, Germany
2006

Workshop „Actinomyceten im Innenraum"
**Groth I**
Jena, Germany
2006

Workshop on "Top-down Approaches in Systems Biology"
**Guthke R**, Pfaff M
Jena, Germany
2006

Anke T, Bringmann G, Francke W, **Grabley S**, **Hertweck C**, Skuballa W, Zeeck A
18. Irseer Naturstofftage, Aktuelle Entwicklungen in der Naturstoff-Forschung
Irsee, Germany
February 2006

2nd ILRS Group Seminar (2007)
**Schmidt D**, **Brakhage AA**
Jena, Germany
2007

3rd International Workshop on "Thrombotic Microangiopathies"
**Zipfel PF**, Misselwitz J, Kentouche K, Wolf G
Jena, Germany
2007

DFG-SPP 1160
Integrated functional genome analysis of human-pathogenic fungi: data warehouse for the integration of transcriptome-, proteome- and metabolome data
Kick Off Meeting
**Guthke R**
Jena, Germany
2007

Workshop "Data and Knowledge Based Biomolecular Network Reconstruction"
**Guthke R**, Pfaff M
Jena, Germany
2007

NiSIS / JCB / DFG International Spring School "Data Mining and Modelling in Systems Biology: Integrative Analysis of Transcriptome and Proteome Data"
**Guthke R**, Pfaff M
Jena, Germany
March 2007

NiSIS / JCB / DFG International Workshop "Data Mining and Modelling in Systems Biology: Data and Knowledge Based Biomolecular Network Reconstruction"
**Guthke R**, Pfaff M
Jena, Germany
March 2007

1st International Workshop "Theragnostics on Severe Infection"
**Brakhage AA**, Reinhart K, Straube E
Weimar, Germany
September 2007

1st ILRS Symposium of the International Leibniz Research School
**Brakhage AA**
Dornburg, Germany
September 2007

2nd International Workshop "Theragnostics on Severe Infections Leading to Sepsis"
**Brakhage AA**, Reinhart K, Straube E
Jena, Germany
October 2007

Workshop „Tropfenbasierte Lebend-Zell-Assays für die mikrobiologische Diagnostik"
**Roth M**, **Martin K**
Jena, Germany
October 2007

Colloquium of the DFG-Priority Programme 1160 "Colonisation and Infection by Human-Pathogenic Fungi"
**Brakhage AA**, **Kniemeyer O**
Jena, Germany
November 2007

International Proteomics Workshop of the Signalpath Marie Curie Research Training Network (EU)
**Brakhage AA**, **Kniemeyer O**, **Heinekamp T**
Jena, Germany
December 2007

Anke T, Bringmann G, **Grabley S**, **Hertweck C**, Kirschning A, Skuballa W
19. Irseer Naturstofftage, Aktuelle Entwicklungen in der Naturstoff-Forschung
Irsee, Germany
February 2007

## Participation in Research Networks 2006/2007
## Beteiligung an Netzwerken und Verbundprojekten 2006/2007

### Sonderforschungsbereiche der Deutschen Forschungsgemeinschaft

**Sonderforschungsbereich 604: Multifunktionelle Signalproteine**
Teilprojekt B02:Regulation of DNA polymerase alpha, Cdc45 and TopBP1 at the Initiation step of DNA replication
Frank Hänel
Laufzeit: 07/2005 - 12/2008

### Schwerpunktprogramme der Deutschen Forschungsgemeinschaft

**Schwerpunktprogramm 1160: Kolonisation und Infektion durch humanpathogene Pilze**
Koordination
Axel Brakhage
Laufzeit: 05/2004 – 06/2008

**Schwerpunktprogramm 1160: Kolonisation und Infektion durch humanpathogene Pilze**
Teilprojekt: Identification and characterisation of virulence associated genes during oral infections with *Candida albicans*
Bernhard Hube
Laufzeit: 05/2004 – 04/2007

**Schwerpunktprogramm 1160: Kolonisation und Infektion durch humanpathogene Pilze**
Teilprojekt: Immune evasion mechanisms of the human pathogenic yeast *Candida albicans*
Peter Zipfel
Laufzeit: 05/2004 – 04/2006

**Schwerpunktprogramm 1160: Kolonisation und Infektion durch humanpathogene Pilze**
Teilprojekt: Studies on the differential expression of *Candida albicans* genes during early infection of Porcine Intestinal Epithelium (PIE) and functional analysis of the target genes
Jürgen Wendland
Laufzeit: 06/2004 – 07/2006

**Schwerpunktprogramm 1160: Kolonisation und Infektion durch humanpathogene Pilze**
Teilprojekt: Metabolism and morphogenesis of human pathogenic fungi
Matthias Brock
Laufzeit: 10/2004 – 08/2006

**Schwerpunktprogramm 1160: Kolonisation und Infektion durch humanpathogene Pilze**
Teilprojekt: Identification of virulence determinants of the human-pathogenic Fungus *Aspergillus fumigatus* by proteome analysis
Axel Brakhage
Laufzeit: 02/2005 – 02/2007

**Schwerpunktprogramm 1152: Evolution metabolischer Diversität**
Teilprojekt: Evolution und Funktion von cis-/trans-Elementen pilzlicher Sekundärmetabolismus-Gene am Beispiel der Penicillinbiosynthese
Axel Brakhage
Laufzeit: 06/2005 – 06/2007

**Schwerpunktprogramm 1150: Signalwege zum Zytoskelett und bakterielle Pathogenität**
Teilprojekt: Analyses of G-protein mediated signals on the organization of the actin cytoskeleton, polar cell growth and the pathogenicity of *Candida albicans*
Jürgen Wendland
Laufzeit: 08/2005 – 12/2006

**Schwerpunktprogramm 1152: Evolution metabolischer Diversität**
Teilprojekt: Die nicht-colinearen Aureothin- und Neoaureothin-Biosynthesewege als Modellsystem für die Evolution von Polyketidsynthasen
Christian Hertweck
Laufzeit: 02/2006 – 09/2007

**Schwerpunktprogramm 1160: Kolonisation und Infektion durch humanpathogene Pilze**
Teilprojekt: Immune evasion mechanisms of the human pathogenic yeast *Candida albicans*
Peter Zipfel
Laufzeit: 07/2006 – 06/2008

**Schwerpunktprogramm 1160: Kolonisation und Infektion durch humanpathogene Pilze**
Teilprojekt: Identification and characterisation of virulence associated genes during oral infections with *Candida albicans*
Bernhard Hube
Laufzeit: 07/2006 – 06/2008

**Schwerpunktprogramm 1160: Kolonisation und Infektion durch humanpathogene Pilze**
Teilprojekt: Molecular analysis of the poly-telomeric CTA2 gene family of *Candida albicans* Elucidation of genetic or epigenetic regulation of their differential expression, localisation and functional analyses *in vitro* and during the early phase of colonisation on porcine intestinal epithelium
Jürgen Wendland
Laufzeit: 07/2006 – 06/2008

**Schwerpunktprogramm 1160: Kolonisation und Infektion durch humanpathogene Pilze**
Teilprojekt: Identification of virulence determinants of the human-pathogenic Fungus *Aspergillus fumigatus* by proteome analysis
Axel Brakhage
Laufzeit: 08/2006 – 02/2009

**Schwerpunktprogramm 1160: Kolonisation und Infektion durch humanpathogene Pilze**
Teilprojekt: Holistic approach to genomics of human-pathogenic fungi: Data warehouse for integration of data on transcriptome, proteome and metabolome of *Candida albicans* and *Aspergillus fumigatus*
Axel Brakhage/Reinhard Guthke
Laufzeit: 10/2006 – 09/2008

### EU-Projekte

**Schwerpunkt: Quality of Life and Management of Living Resources (FP5)**
Teilprojekt: Eukaryotic polyketides in surrogate hosts
Christian Hertweck
Laufzeit: 11/2002 – 07/2006

**Marie Curie Research Training Networks**
Teilprojekt: Interaction of fungal pathogens with host cells: a post-genomic approach GALAR FUNGAIL 2
Bernhard Hube
Laufzeit: 01/2004 – 12/2007

**Programme Information Society Technologies FP6/2002/IST/1**
Teilprojekt: Nature-inspired Smart Information Systems
Reinhard Guthke
Laufzeit: 01/2005 – 01/2008

**Marie Curie Research Training Networks SIGNALPATH (FP6)**
MAP kinase cascades controlling virulence in fungi: from signals to pathogenicity response
Axel Brakhage
Laufzeit: 11/2005 – 10/2009

**Integrated Project: New medicines for tuberculosis (NM4TB) (FP6)**
Ute Möllmann
Laufzeit: 01/2006 – 12/2008

**Integrating and strengthening the European Research Area Specific Targeted Project (MANASP)**
Development of novel management strategies for invasive aspergillosis
Axel Brakhage
Laufzeit: 12/2006 – 11/2009

### Verbundvorhaben des Bundesministeriums für Bildung und Forschung

**Verbundvorhaben: JCB (Jenaer Centrum für Bioinformatik)**
Teilprojekt C.2: Nachwuchsgruppe Mustererkennung in gesunden und kranken menschlichen Zellen
Teilprojekt D.1.A: Vorhersage und Analyse komplexer molekularer Interaktionen von genregulierten Netzwerken in menschlichen Zellen
Reinhard Guthke
Laufzeit: 09/2001 – 06/2007

**Verbundvorhaben "MONACO" Hochdurchsatz-Monitoring zur Funktionalen Analyse von Naturstoffen**
Teilprojekt 1: Neue biologische aktive Substanzen aus chinesischen Heilpflanzen und Schwämmen und ihren assoziierten Mikroorganismen
Susanne Grabley
Laufzeit: 04/2003 – 12/2006

**Verbundvorhaben: Sysbio-Plattform Zellbiologie: Dreidimensionale bioartifizielle humane Leberzellsysteme**
Teilprojekt 3: Quantitative Charakterisierung der Dynamik von Leberzell-Populationen im 3D-Leberzell-Bioreaktor
Reinhard Guthke
Laufzeit: 01/2004 – 12/2006

**Verbundvorhaben MN V: Marine Pilze II, Kultivierung (A); Leitantrag; Vorhaben: Charakterisierung und Evaluierung von neuen Wirkstoffen für die Krebstherapie und die Behandlung von Therapie-resistenten bakteriellen Infektionen**
Teilprojekt A: Kultivierung und Wirkstoff-Identifizierung
Isabel Sattler
Laufzeit: 04/2004 – 09/2006

**Kompetenznetzwerk: Genomforschung an Bakterien für den Umweltschutz, die Landwirtschaft und die Biotechnologie**
Teilprojekt: Analyse der Biosynthese gemischter Sekundär-Metabolite in Streptomyceten
Christian Hertweck/Dirk Schwartz
Laufzeit: 06/2004 – 08/2006

**Verbundvorhaben: BioChancePLUS Knochenersatzmaterialien zur Therapie der Osteoporose**
Teilprojekt: Entwicklung und Optimierung der gentechnischen Produktion von rhBMP-2 und rhBMP-14 (MP52); BMP-Modifizierung der Knochenersatzmaterialien und Implantate; Entwicklung injizierbarer rhBMP-Suspensionen; in vitro

Evaluierung der BMP-Freisetzung von Trägermaterialien und Bestimmung der BMP-Oberflächenaktivität und Testung der biologischen Aktivität der BMP-modifizierten Materialien in Zellkultur
Peter Hortschansky
Laufzeit: 07/2004 – 06/2007

**Verbundprojekt Hochdurchsatz-Bioassay-System auf Basis mikroserieller Zellkulturen in flüssig/flüssig-Zweiphasensystemen SERIZELL**
Teilvorhaben: Systemevaluierung mit ausgewählten Bioassays
Martin Roth, Karin Martin
Laufzeit: 01/2005 – 12/2007

**Verbundvorhaben GenomikPlus**
Teilprojekt: Neue antibakterielle und antitumorale Polyketide durch Biokombinatorik
Christian Hertweck
Laufzeit: 08/2006 – 07/2009

**Verbundvorhaben: BioChancePLUS-2: Verbundprojekt: Individualisierte Medizin: Tool zur Therapieentscheidung – Apherese/Immunadsorption-Chip**
Teilprojekt 4: Bioinformatische Analysen der RA-Patienten: Identifizierung von Genen und Genprodukten für das Ansprechverhalten zur Apherese-Immunadsorptionstherapie
Reinhard Guthke
Laufzeit: 09/2006 – 08/2009

**Verbundvorhaben: HepatoSys-Systembiologie regenerierender Hepatozyten**
Teilprojekt: B3: Dynamische Modellierung des Wnt/beta-Catenin Signalweges während der Leberregeneration
Reinhard Guthke
Laufzeit: 01/2007 – 12/2009

### Weitere Verbundvorhaben

**Verbundvorhaben: Focusing on Hormone-Independent Breast Cancer**
**German Israeli Foundation for Scientific Resaerch & Development**
Christian Hertweck
Laufzeit: 01/2006 – 12/2008

**Verbundvorhaben: Novel Derivatization / Functionalization of Natural Products**
**National Institutes of Health (NIH, USA)**
Ute Möllmann
Laufzeit: 09/2005 – 07/2008

**Verbundvorhaben: Untersuchungen zum Vorkommen und zur gesundheitlichen Relevanz von Bakterien in Innenräumen**
**Bundesumweltamt**
Ingrid Groth, Karin Martin
Laufzeit: 12/2005 – 05/2008

**Verbundvorhaben: Zellex-Einzel-Zell-Identifikation und Einzel-Zell-Gen-Expressionsdiagnostik durch in-situ RT-PCR in Mikrodurchflußreaktoren**
**Thüringer Kultusministerium**
Teilprojekt: Typing von Mikroorganismen mittels PCR
Hans-Peter Saluz
Laufzeit: 01/2004 – 12/2006

## Calls for Appointments 2006/2007
## Rufe 2006/2007

**Hertweck, Christian** (2006)
Professor für Naturstoffchemie
Biologisch-Pharmazeutische und Chemisch-Geowissenschaftliche Fakultät der Friedrich-Schiller-Universität Jena

**Hube, Bernhard** (2006)
Lehrstuhl für Mikrobielle Pathogenität
Biologisch-Pharmazeutische Fakultät der Friedrich-Schiller-Universität Jena

**Wendland, Jürgen** (2006)
Professor for Yeast Biology
Carlsberg Research Center, Copenhagen, Denmark

**Schmitt, Imke** (2007)
Assistant Professor for Plant Biology
University of Minnesota, St. Paul, USA

## Postdoctoral Lecture Qualifications 2006/2007
## Habilitationen 2006/2007

**Hertweck, Christian** (2006)
Über das Verständnis der mikrobiellen Polyketidbiosynthese zu neuen Wirkstoffen
Friedrich-Schiller-Universität Jena

## Graduations 2006/2007
## Promotionen 2006/2007

### 2006

**Heinen, Stefan**
Die Rolle von Faktor H bei atypischem hämolytisch urämischen Syndrom (aHUS) und der membranoproliferativen Glomerulonephritis Typ 2 (MPGN II)
Friedrich-Schiller-Universität Jena

**Kemami Wangun, Hilaire Vignie**
Isolation, Structure Elucidation and Evaluation of Anti-inflammatory and Anti-infectious Activities of Fungal Metabolites
Friedrich-Schiller-Universität Jena

**Li, Liya**
Secondary metabolites from mangrove plant *Hibiscus tiliaceus* and its related microbionts
HKI and Peking University

**Loos, Sabine**
Polyketidsynthasegene aus *Penicillium simplicissimum* und Strategien zur Funktionsanalyse
Friedrich-Schiller-Unversität Jena

**Rassmann, Alexander**
Aufklärung von Coxsackievirus B3-vermittelten Pathogeneseprozessen durch molekulare Analyse von zweidimensionalen Proteinmustern.
Friedrich-Schiller-Universität Jena

**Traitcheva, Nelly**
Molecular Analysis of the Neoaureothin Biosynthesis Gene Cluster from *Streptomyces orinoci* HKI 0260: a Model System for the Evolution of Bacterial Polyketide Synthases
Friedrich-Schiller-Universität Jena

**Ußkilat, Christian**
Das Topoisomerase-IIβ-bindende Protein 1: Untersuchungen zur Regulation des Genpromotors und Funktion des Proteins.
Friedrich-Schiller-Universität Jena

**Wangun, Hilaire Kemami**
Isolierung, Strukturaufklärung und Derivatisierung von Metaboliten aus Pilzen
Friedrich-Schiller-Universität Jena

**Xu, Minjuan**
Secondary metabolites from mangrove plant *Aegiceras corniculatum* and endophytic fungus *Penicillium* sp.
HKI and Peking University

**Xu, Zhongli**
The Biosynthesis of Benastatin and Chartreusin, Two Structurally Intriguing Aromatic Polyketides
Friedrich-Schiller-Universität Jena

### 2007

**Carlsohn, Marc Rene**
Molekulare Taxonomie seltener Aktinomyceten aus schwermetallhaltigen Habitaten
Friedrich-Schiller-Universität Jena

**Dünkler, Alexander**
RhoGTPasen in *Candida albicans* und deren Einfluss auf das polarisierte Wachstum und die Zytokinese
Friedrich-Schiller-Universität Jena

**Friedrich, Herdis**
Untersuchungen zu mikrobiellen Interaktionen von Pilzen der Gattung *Phoma*.
Friedrich-Schiller-Universität Jena

**Kraft, Mario**
Duale Optimierung der Darstellung heterologer Proteine in *Escherichia coli*: Parallele und differenzielle Produktion von Centromerproteinen des inneren humanen Kinetochors.
Friedrich-Schiller-Universität Jena

**Maerker, Claudia**
Die zwei metabolischen Funktionen der Aconitase AcoA aus *Aspergillus nidulans*: Aconitase-Aktivität im $[4Fe\text{-}4S]^{2+}$ und Methylisocitrate-Dehydratase-Aktivität im $[3Fe\text{-}4S]^{+}$ Zustand
Leibniz Universität Hannover

**Martin, Ronny**
Molekulare Analyse des Aktinzytoskeletts in *Candida albicans*
Friedrich-Schiller-Universität Jena

**Mihatsch, Katharina**
Characterisation of viral pathogenic mechanisms on the basis of interactions of multifunctional coxsackievirus/host protein complexes
Friedrich-Schiller-Universität Jena

**Partida-Martinez, Laila Pamela**
Discovery of endofungal bacteria: new insights into toxin biosynthesis and bacterial-fungal symbiosis
Friedrich-Schiller-Universität Jena

**Schenk, Tino**
Charakterisierung des humanen Tumormarkers PRAME
Friedrich-Schiller-Universität Jena

**Wilson, Duncan**
The role of phosphodiesterases in *Candida albicans* physiology
University of Manchester

**Winkler, Robert**
Biosynthese von Nitroverbindungen: Charakterisierung der ungewöhnlichen Mangan-abhängigen N-Oxygenase AurF
Friedrich-Schiller-Universität Jena

**Scherlach, Kirstin**
Neue Wege zu antitumoralen Naturstoffen aus Pflanzen und Mikroorganismen
Friedrich-Schiller-Universität Jena

**Göllner, Stefanie**
Untersuchung der transkriptionellen Response von *Chlamydophila psittaci* im Stadium der induzierten Resistenz
Friedrich-Schiller-Universität Jena, FLI

## Diploma Theses 2006/2007
## Diplomarbeiten 2006/2007

### 2006

**Albrecht, Daniela**
Einbindung einer Proteom-Transkriptom-Datenbank in das lokale Rechnernetz des HKI sowie Konfiguration für spezielle mikrobiologische Experimente
Friedrich-Schiller-Universität Jena

**Flores de Looß, Carina**
Rhizonin, the first isolated mycotoxin from lower fungi is produced by symbionts
Friedrich-Schiller-Universität Jena

**Gropp, Katharina**
Die Rolle der EGR-Proteine im TLR-Weg
Friedrich-Schiller-Universität Jena

**Hecker, Michael**
Modellierung der Genregulation von Zytokinen während der antirheumatischen Therapie mit einem TNF-α-Blocker
Friedrich-Schiller-Universität Jena

**Kreutzer, Stefanie**
Klonierung und Expression von eukaryotischen Luciferasegenen für die Quantifizierung von Promotorstärken in *Streptomyces lividans*
FH Jena

**Kusebauch, Björn**
Untersuchungen zur Synthese und Biogenese von Orinocin und verwandten Pyronverbindungen
Friedrich-Schiller-Universität Jena

**Lackner, Gerald**
Molekularbiologische Untersuchung und Manipulation der Biosynthese des aromatischen Polyketids Benastatin
Friedrich-Schiller-Universität Jena

**Lauer, Nadine**
Funktionelle Bedeutung der bei der altersabhängigen Maculadegeneration des Auges beschriebenen Faktor H bzw. FHL-1 Mutation H402Y
Friedrich-Schiller-Universität Jena

**Lokotsch, Josephine**
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**Lucas, Jörg**
Neurodegenerative Krankheiten und Apoptose: potenzielle Targets basierend auf Protein/Protein-Interaktionen
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**Möhle, Niklas**
Herstellung und Analyse von Mutanten der cAMP-Signaltransduktion von *Aspergillus fumigatus*
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**Reuter, Michael**
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**Schindler, Susann**
Charaktisierung FH interagierender Proteine
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**Schöbel, Felicitas**
Charakterisierung von Schlüsselenzymen der Lysinbiosynthese und des Glyoxylatzyklus aus *Aspergillus fumigatus*
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**Schünemann, Antje**
Klonierung, Expression und funktionelle Charakterisierung der Kohlenhydrat modifizierenden Enzyme WbpB und WbpK aus *Pseudomonas aeruginosa*
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**Strobel, Stefanie**
Charakterisierung von Autoantikörpern bei HUS und MPGNII
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**Thümmler, Alexander**
Online-*in-vivo*-Monitoring der wachstumsabhängigen Genexpression in *Streptomyces lividans* in Batch- und Chemostat-Kulturen
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**Ulbrich, Berit**
Verbesserungen der Tumorklassifikation anhand molekularbiologischer Muster mit neuen Methoden des unüberwachten Lernens
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Entwicklung eines Fermentationsverfahren zur Herstellung von Cervimycin K
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### 2007

**Borth, Nicole**
Molekulare Untersuchungen von Wal1-Domänen in *Candida albicans* und Funktionsanalysen von CaVRP1
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**Dahms, Sven**
Verifizierung von Zweihybrid-Interaktionssystemen durch Expression und Analyse von Fusionsproteinen mit interner Leserasterverschiebung.
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**Feige, Jens**
Schnelle Pathogendiagnostik in marinen Krustentieren
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Charakterisierung neuer potentieller Tumormarker für AML
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Die Proteinkinase A von *Aspergillus fumigatus*: Identifizierung potentieller Zielproteine
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Untersuchungen zur Prognose von Erkrankungen durch gemeinsame Auswertung von Genexpressionsdaten und Überlebenszeitdaten
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**Li, Xiaoli**
Characterization of a chlamydial protein in the respect of apoptosis inhibition
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**Litsche, Katrin**
Untersuchung der Wirkung bioaktiven *Phellinus*-Pilzextrakts auf Protein- und microRNA-Expression in menschlichen Tumorzellen
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**Marx, Cathleen**
Untersuchung der Vorteile neuer Methoden für die unscharfe Clusteranalyse in der funktionellen Genomanalyse und biomedizinischen Forschung
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**Reiche, Dorit**
Caspase-8 als potenzielles Target der Apoptose-Inhibition durch *Chlamydia trachomatis* und *Chlamydia pneumoniae*
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**Richter, Julia**
Charakterisierung von Komplement-Inhibitoren von pathogenen Organismen
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**Schirmer, Sylvia**
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**Schneider, Thomas**
BCL6 als potentieller Transcriptionsrepressor des humanen Tumormarkers PRAME
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**Schwarze, Daniel**
Ein neuer Weg zur sehr schnellen Identifikation von Pathogenen
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**Song, Shu-Ping**
Studies on four potential chlamydial apoptosis inhibitor candidates
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**Stippa, Selina**
Interaktion von Akut-Phase-Proteinen und Komplementproteinen
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**Vater, Sandra**
Charakterisierung zweier konstitutiver Allele der Gα-Untereinheit GPAB in *Aspergillus fumigatus*
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**Zähle, Christoph**
Identifizierung antifungaler Substanzen aus *Streptomyces microflavus* (HKI 0508) und *Amycolatopsis nigrescens* (HKI 0329)
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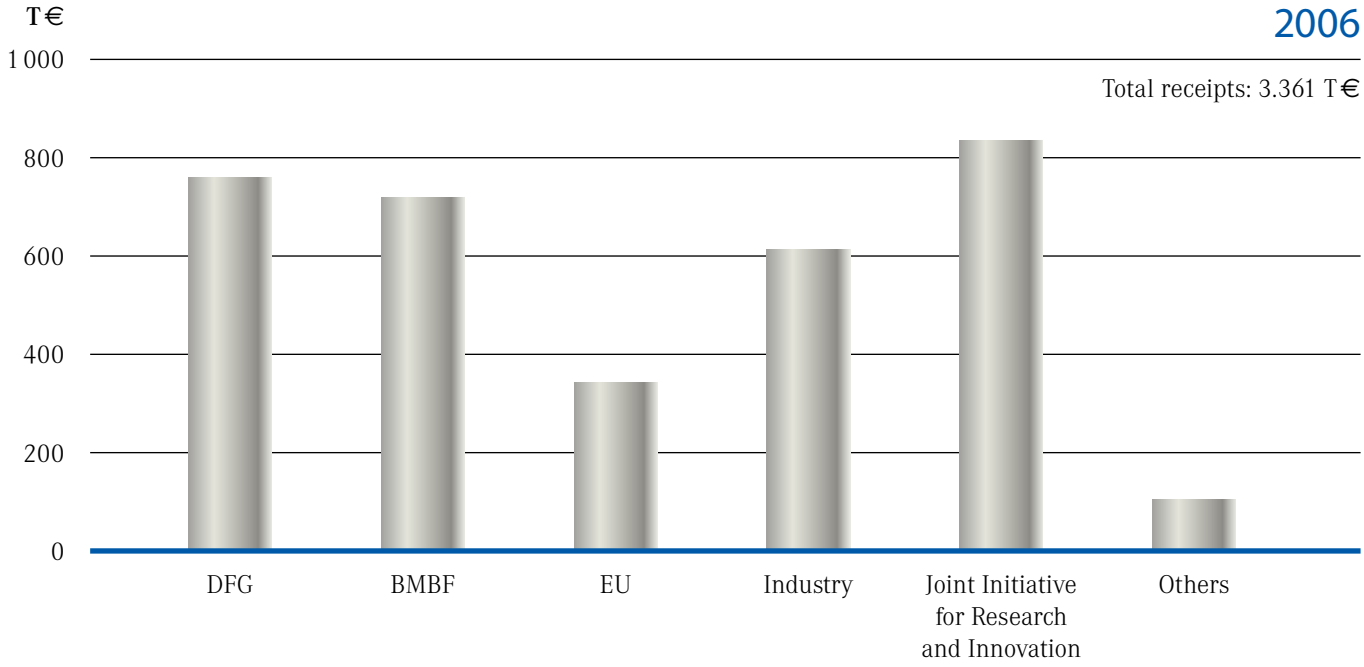
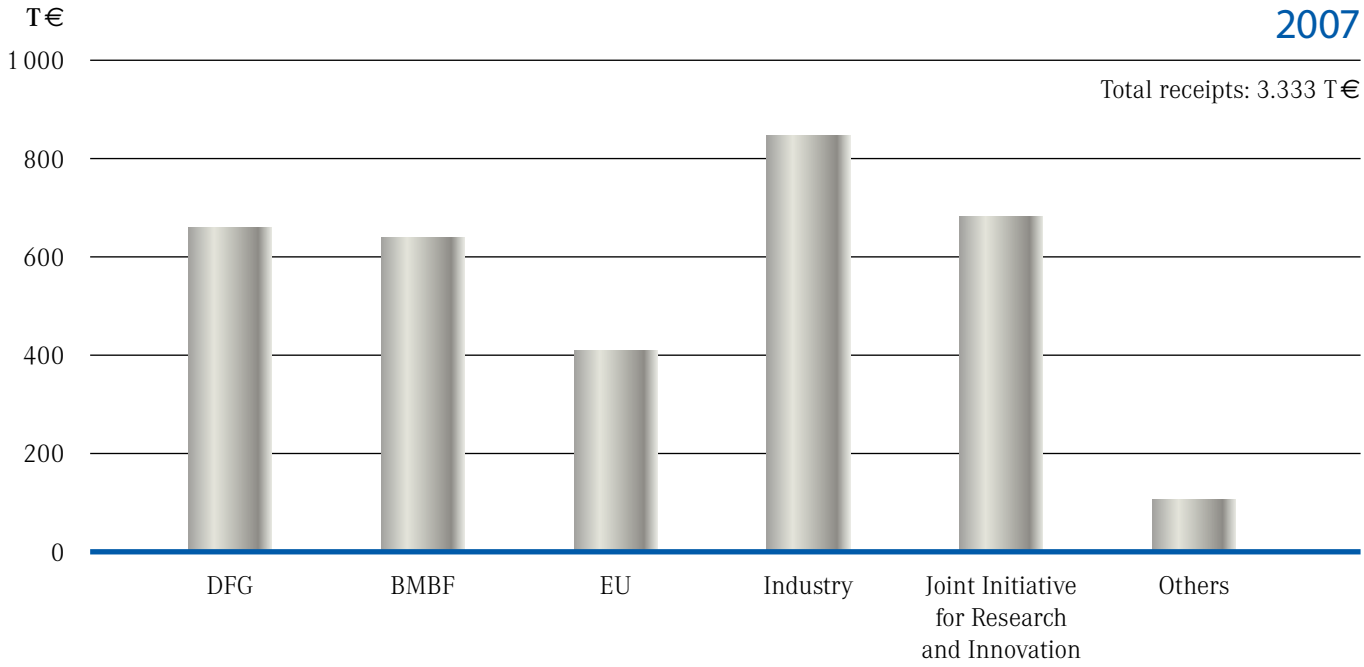
# External Funding 2006/2007
## Drittmittel 2006/2007

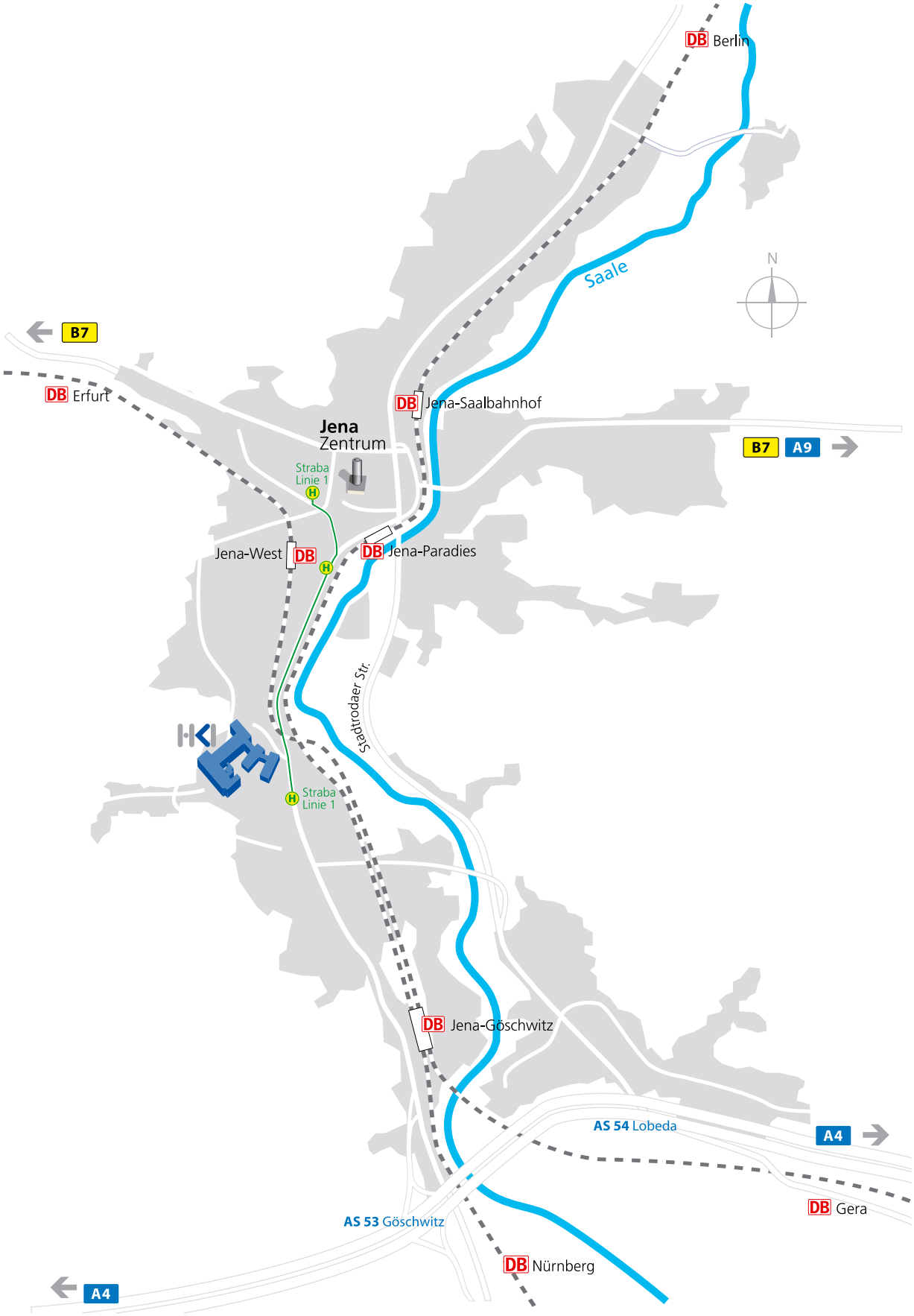


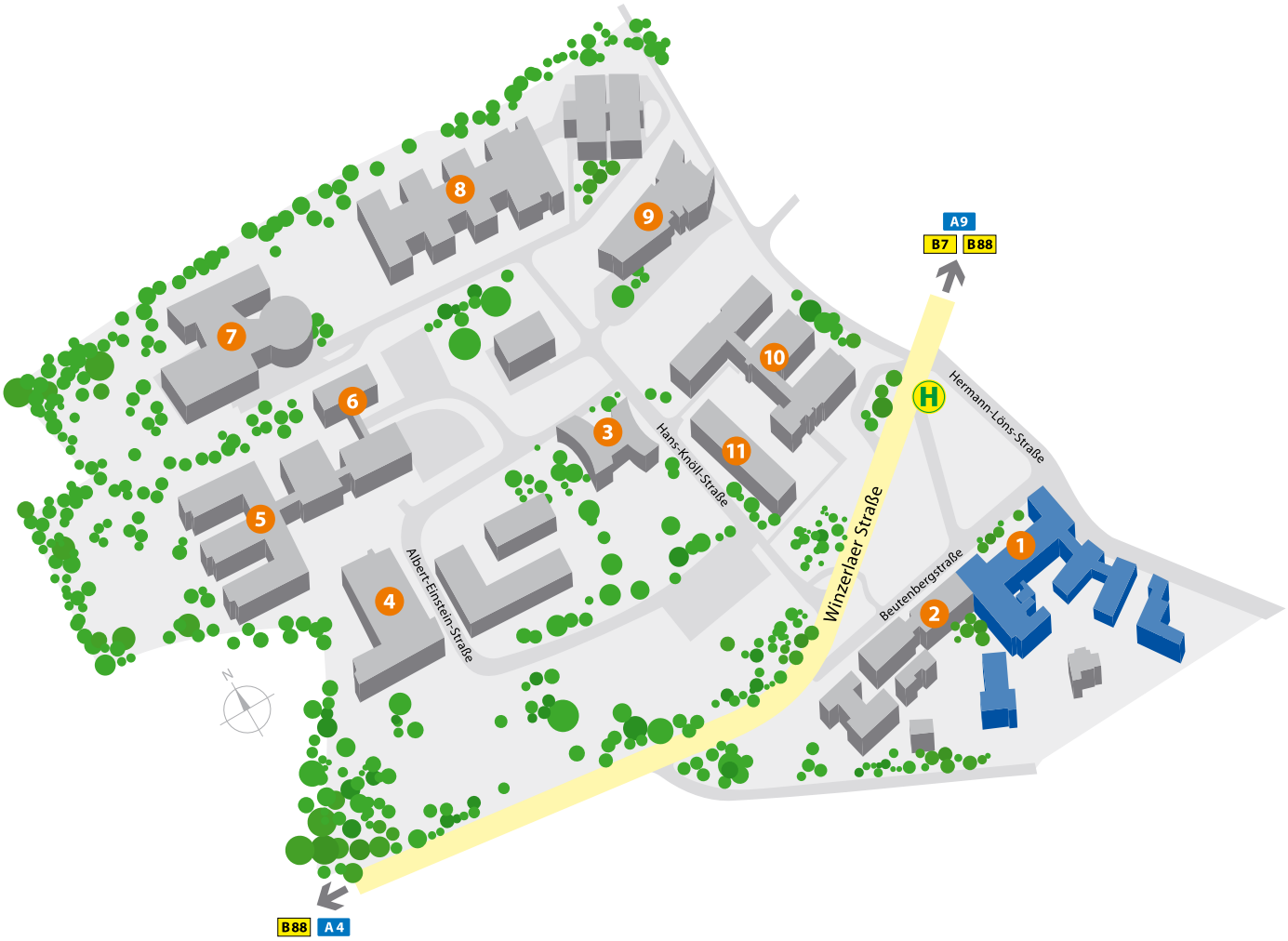




Third-party funds during the period reported: composition of the sources of income
Drittmittelausgaben im Berichtszeitraum: Verteilung über die Drittmittelgeber

# Maps

## Lagepläne



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