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

RESEARCH REPORT 2004/2005

HKI Research Report 2004/2005

Several new developments made 2005 an especially exciting year for the HKI. Professor Axel Brakhage has been the chair of Microbiology and Molecular Biology at the Friedrich-Schiller-University Jena since December, 2004. Also since 2005, he has headed both the Department of Molecular and Applied Microbiology at the HKI and served as Director of the Institute. Accepting the position as Director was facilitated by the substantial efforts, decisions and foresight of the former Directors, Profs. Albert Hinnen and Wolfgang Knorre, who successfully positioned the HKI in the scientific community. In 2005, the HKI changed its name to the Leibniz Institute for Natural Product Research and Infection Biology – Hans Knoell Institute (HKI) to more accurately reflect and document both the further strengthening of infection biology, as proposed by the Wissenschaftsrat 1999, and its membership of the Leibniz Association.

Based on the evaluation of the Wissenschaftsrat in 1999, the departments of Molecular Natural Products Research and Biostructure Chemistry were merged to form the new department Biomolecular Chemistry. Dr. Christian Hertweck became the head of the newly established department. Dr. Hertweck was also concurrently appointed to the position of Chair of Natural Product Chemistry at the Friedrich-Schiller-University Jena.

Dr. Uwe Horn was appointed as the new head of the Bio Pilot Plant. We are also pleased to report that several of our junior research group leaders have advanced their careers and the prestige of the HKI by additional ap-

INTRODUCTION | VORWORT

2005 markiert ein ereignisreiches Jahr für das Leibniz-Institut für Naturstoff-Forschung und Infektionsbiologie – Hans-Knöll-Institut (HKI) mit einer Reihe prägender Veränderungen. Nach der im Dezember 2004 erfolgten Berufung von Professor Dr. Axel A. Brakhage auf den Lehrstuhl für Mikrobiologie und Molekularbiologie an der Friedrich-Schiller-Universität Jena übernahm er im Laufe des Jahres 2005 zunächst die Leitung der Abteilung für Molekulare und Angewandte Mikrobiologie und wurde anschließend zum Direktor des Instituts ernannt. Letztere Aufgabe wurde ganz wesentlich durch die Tatsache erleichtert, daß die vorangegangenen Direktoren, die Professoren Albert Hinnen und Wolfgang A. Knorre das HKI in der Wissenschaftslandschaft hervorragend positioniert und wegweisende Entscheidungen getroffen hatten.

Im Jahr 2005 erfolgte die Umbenennung des Instituts in Leibniz-Institut für Naturstoff-Forschung und Infektionsbiologie – Hans-Knöll-Institut (HKI). Im neuen Namen widerspiegelt sich sowohl die vom Wissenschaftsrat 1999 empfohlene stärkere Hinwendung zu infektionsbiologischer Forschung, als auch die feste Verankerung in der Leibniz-Gemeinschaft.

Im Resultat der Evaluierung durch den Wissenschaftsrat im Jahr 1999 erfolgte eine Zusammenführung der beiden Abteilungen Biostrukturchemie und Molekulare Naturstoff-Forschung zur neuen Abteilung Biomolekulare Chemie. Der neu berufene Lehrstuhlinhaber für Naturstoffchemie an der Friedrich-Schiller-Universität Jena, Professor Dr. Christian Hertweck, konnte als Leiter der Abteilung Biomolekulare Chemie gewonnen werden. Eine in Nature publizierte Arbeit von Christian Hertweck und seiner Mitarbeiterin Laila P. Partida-Martinez über die Wirkstoffbildung durch bakterielle Endosymbionten in Pilzen belegt eindrucksvoll die Leistungsfähigkeit der neuen Abteilung.

Das Biotechnikum wird seit 2005 von Dr. Uwe Horn geleitet. Für die erfolgreiche Entwicklung einer Technologie zur Isolierung

pointments, including offers of Professorships or industrial positions to Drs. Schwartz, Hertweck and Hellwage.
Also in 2005, under the auspices of the „Pakt für Forschung und Innovation" the HKI obtained a grant to establish the first Leibniz Graduate School designated "International Leibniz Research School for Microbial and Biomolecular Interactions (ILRS Jena)". It was founded with partner Institutes of the Friedrich-Schiller-University, Jena and the Max Planck Institute for Chemical Ecology. The graduate school started in 2006, as scheduled.

In the summer of 2005, the former Lab building 3 was rebuilt. It was dedicated and reopened by the Ministry of Culture, Professor J. Goebel. The building now houses teaching facilities devoted to summer schools and meetings, as well as a modern laboratory tract for the new department Microbial Pathogenicity Mechanisms which will be established in 2006/2007.

Scientifically, the HKI identifies and investigates natural products from a variety of sources, and especially those synthesised by microorganisms, to understand their role as mediators of biological interactions, to use them for the elucidation of cellular processes and to identify their potential biological activity. These investigations include screening for biological activity as well as the production and structure elucidation of novel natural products. Within the framework of the "Internal Product Line", the HKI incorporates

und Analyse mikrobieller Einzelzellkulturen wurde den Mitarbeitern des Technikums Martin Roth und Karin Martin gemeinsam mit Kooperationspartnern aus drei weiteren Instituten der Thüringer Forschungspreis 2005 in der Sparte Angewandte Forschung verliehen. Das gemeinsame Berufungsverfahren mit der Friedrich-Schiller-Universität für die Besetzung des Lehrstuhls Mikrobielle Pathogenität und für die am HKI neu zu etablierende Abteilung Mikrobielle Pathogenitätsmechanismen wurde eingeleitet.
Besonders erfreulich ist die Entwicklung der Nachwuchsgruppen. So erhielten Dr. Dirk Schwartz, Dr. Christian Hertweck und Dr. Jens Hellwage Rufe auf Professuren oder besetzten hochqualifizierte Industriepositionen. Sie stehen damit exemplarisch für den Erfolg des Nachwuchsgruppen-Konzeptes bei der gezielten Förderung hochqualifizierter junger Wissenschaftler.
Ebenfalls 2005 erhielt das HKI im Rahmen des Paktes für Forschung und Innovation den Zuschlag für die Gründung der ersten Graduiertenschule der Leibniz-Gemeinschaft. Die „International Leibniz Research School for Microbial and Biomolecular Interactions" (ILRS Jena) wurde gemeinsam mit Partnerinstitutionen an der Friedrich-Schiller-Universität Jena und dem Max-Planck-Institut für chemische Ökologie ins Leben gerufen und beginnt im Frühjahr 2006 mit der Rekrutierung der ersten Doktoranden, die mehrheitlich aus dem Ausland stammen werden.
Im Sommer 2005 konnte der Um- und Erweiterungsbau des Laborhauses 3 mit der Eröffnung durch den Thüringer Kultusminister Prof. Dr. Jens Goebel abgeschlossen werden. Das auch architektonisch hochmoderne Gebäude enthält Kursräume für internationale Sommerschulen, Tagungen und Workshops sowie einen modernen Labortrakt für die neu zu etablierende Abteilung Mikrobielle Pathogenitätsmechanismen.
Das HKI hat sich aktiv an Anträgen im Rahmen der Exzellenzinitiative der DFG beteiligt.

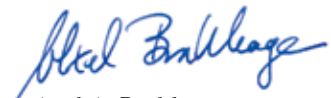
an extensive array of over 25 assays to determine the scope of activity of natural products, derivatives and analogs which are also prepared by our internal teams and through extensive external collaborations that are a characteristic strength of the HKI. Moreover, molecular genetic techniques have been used to analyze, modify and identify novel microbial, especially fungal, gene clusters which, in many cases, are silent. Activation of silent gene clusters has been achieved by genetic engineering. As described below, these studies guide and complement further developments related to the goals of the HKI. The analysis of regulatory mechanisms involved in the biosyntheses of natural products helps elucidate the evolution and physiological role of the compounds and also contributes to improved production. Furthermore, scientists at the Institute are interested in understanding the pathobiology of human-pathogenic fungi and developing novel natural product-based antibiotics mainly directed against pathogenic fungi. Some of these compounds, when produced by pathogenic fungi, act as virulence determinants. Genomic techniques such as proteome, transcriptome and metabolome analyses not only facilitate an understanding of the complex interaction of pathogens and hosts, but also define the cellular conditions required for the biosyntheses of natural products. Bioinformatic analyses, such as pattern recognition, help to generate holistic models (systems biology) that further complement the goals of the HKI.

Der von Analytik Jena in Kooperation mit dem HKI entwickelte SpeedCycler für die ultraschnelle Amplifikation von DNA ist in den Markt eingeführt worden und kommerziell erhältlich. Sehr erfreulich ist auch, dass die vom HKI ausgegründete Firma Prothera von WackerChemie übernommen wurde und jetzt als WackerBiotech den Wissenschaftsstandort Jena weiter stärkt.

Wissenschaftlich beschäftigt sich das HKI mit allen Aspekten der Naturstoff-Forschung hauptsächlich an Mikroorganismen. Dazu zählen klassisches Screening, die Produktion und Strukturaufklärung von Naturstoffen sowie die Erforschung der potenziellen pharmakologischen Eigenschaften dieser Verbindungen. Das HKI ist im Rahmen der Durchgehenden Bearbeitungslinie in der Lage, alle klassischen, für die Aufklärung und Charakterisierung von Naturstoffen erforderlichen Untersuchungen durchzuführen. Zusätzlich beschäftigen sich Arbeitsgruppen mit molekulargenetischen Ansätzen, die auf der Verfügbarkeit von Genomen von naturstoffproduzierenden Bakterien und Pilzen beruhen (Clusteridentifizierung und -aktivierung, kombinatorische Biosynthese) sowie auch der Analyse molekularer Regulationsmechanismen von Genclustern des Sekundärmetabolismus und der Evolution solcher Cluster. Den zweiten großen Schwerpunkt des Instituts bildet die infektionsbiologische Forschung, die in erster Linie human-pathogenen Pilzen und deren Interaktion mit dem Wirt gewidmet ist. Die Expertise der Arbeitsgruppen ergänzt sich optimal, da einige Sekundärstoffe auch Virulenzdeterminanten darstellen. Techniken der funktionellen Genomanalyse wie Proteom-, Transkriptom- und Metabolomanalysen tragen zu einem besseren Verständnis der komplexen Wechselwirkungen zwischen Krankheitserrger und Wirtsorganismus bei. Die am HKI intensiv betriebene Datenanalyse mit Methoden der Bioinformatik und Mustererkennung dient in einem systembiologischen Ansatz der Erstellung holistischer Modelle.

Scientists at the HKI also are engaged in education and training of graduates at all levels. In close co-operation with the Friedrich-Schiller-University, Jena, an increasing number of PhD students are advised at the HKI. Embedded in predominantly third-party funded projects, they benefit from the state-of-the-art research facilities at the HKI and the highly integrative network of life science groups at the Beutenberg Campus. Additionally, our scientists are crosslinked in various research projects that have resulted in the generation of nine spin-off companies from the HKI in recent years.

Overall, during 2004 – 2005, the HKI continued its mission, and, through the extensive efforts of its researchers and staff, made significant contributions to both fundamental and applied science that were consistent with its new designation as the Leibniz Institute for Natural Product Research and Infection Biology – Hans Knoell Institute (HKI). The advances made are reflected in extensive publications in the most relevant peer reviewed journals, additional external funding and the recognition of our colleagues by numerous appointments. We look forward to continued growth and success.



Axel A. Brakhage

Die Wissenschaftler des HKI sind in allen Stufen der akademischen Ausbildung engagiert. In enger Kooperation mit der Friedrich-Schiller-Universität wird eine kontinuierlich wachsende Zahl leistungsstarker Doktorandinnen und Doktoranden am HKI betreut. Diese profitieren bei der Bearbeitung ihrer überwiegend drittmittelfinanzierten Projekte von der hochmodernen Ausstattung des Instituts und einem lebendigen Netzwerk von Forschergruppen im Bereich Lebenswissenschaften auf dem Jenaer Beutenberg-Campus. Weiterhin bestehen über verschiedene Projekte enge Kontakte zur Fachhochschule Jena und zu jungen Biotechnologie-orientierten Unternehmen, von denen neun allein aus dem HKI hervorgegangen sind.
Zum Redaktionsschluß des vorliegenden Forschungsberichtes beherbergte das HKI vier Abteilungen: Molekulare und Angewandte Mikrobiologie (Axel A. Brakhage), Infektionsbiologie (Peter F. Zipfel), Zell- und Molekularbiologie (Hans Peter Saluz) sowie Biomolekulare Chemie (Christian Hertweck). Die Einrichtung einer fünften Abteilung zusammen mit der Friedrich-Schiller-Universität Jena als Lehrstuhl „Mikrobielle Pathogenität“ erfolgt zur Zeit. Desweiteren beherbergt das HKI mehrere Nachwuchs- und Forschergruppen, die zum Teil gemeinsame Gruppen mit der FSU darstellen. Als serviceorientierte Einheit mit einem erheblichen Anteil an eigener Forschungskapazität ist das Biotechnikum (Uwe Horn) für alle Abteilungen und Nachwuchsgruppen sowie eine Vielzahl externer Kooperationspartner übergreifend aktiv.

Dem geneigten Leser wünsche ich viel Freude bei der Lektüre unseres Forschungsberichts.

Axel A. Brakhage

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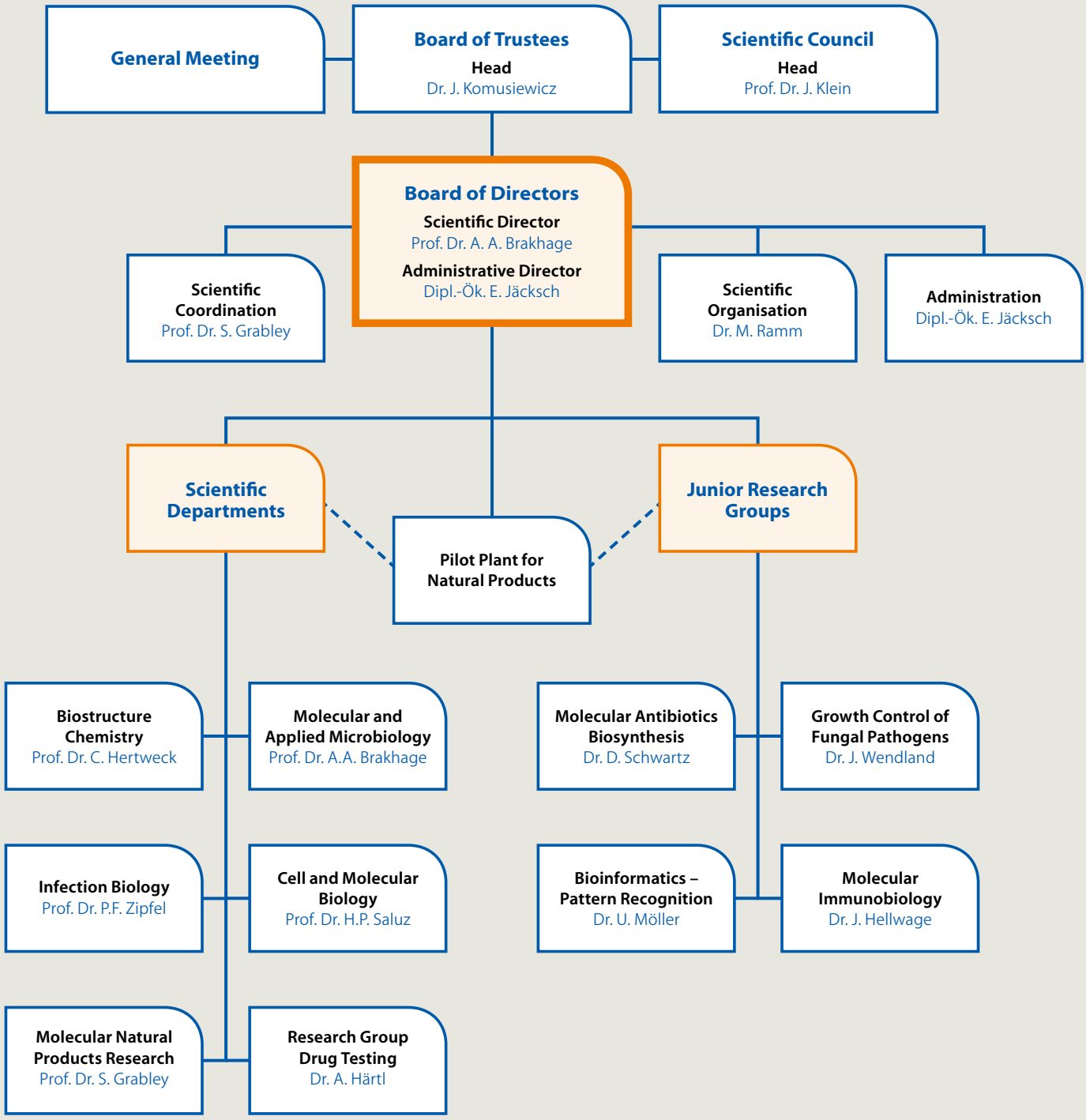
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Department of Biostructure Chemistry and Junior Research Group Bioorganic Synthesis

Department of Biostructure Chemistry and Junior Research Group Bioorganic Synthesis*

The Departments of 'Bioorganic Synthesis' and 'Biostructure Chemistry' (since 2006: 'Biomolecular Chemistry'), headed by Prof. Dr. Christian Hertweck, focus on various aspects of microbial secondary metabolism. In addition to the isolation, structural elucidation and chemical derivatization of bioactive compounds produced by bacteria and fungi, the groups jointly investigate the mechanisms of biosynthetic pathways by a combination of chemical and biological methods. These multidisciplinary projects are integrated into national and international academic competence networks (EU-, BMBF and DFG-funded), many of which are linked to industrial partners. The groups aim at a deeper understanding of the evolution of metabolic diversity, and at exploiting Nature's biosynthetic potential to generate novel natural product derivatives. In particular, biosynthetic pathways of pharmacologically relevant polyketides are investigated. These projects are supported by two EMBO und Humboldt postdoctoral fellows from Finland and Hungary, respectively.

Another important research topic involves the elucidation of microbial pathogenicity factors and their molecular basis. In this regard, the recently discovered phytopathogenic alliance of fungi and endosymbiotic bacteria is of particular interest. The evolution of such multicomponent systems and their microbial and

INTRODUCTION | EINLEITUNG

Head
Prof. Dr. Christian Hertweck

*in 2003 both groups merged into one. Since 2006 the department is entitled "Department of Biomolecular Chemistry"

Die Abteilungen Bioorganische Synthese und Biostrukturchemie (seit 2006: Biomolekulare Chemie) befassen sich unter der Leitung von Prof. Dr. Christian Hertweck mit dem facettenreichen Stoffwechsel von Bakterien und Pilzen. Neben der Strukturaufklärung und der chemischen Derivatisierung von Wirkstoffen aus Mikroorganismen werden die Mechanismen der Biosynthesewege mit chemischen und biologischen Methoden studiert. Diese interdisziplinären Arbeiten werden in regionalen und überregionalen akademischen Netzwerken (größtenteils EU-, BMBF- und DFG-gefördert) und mit Industriebeteiligung durchgeführt. Die Projekte haben nicht nur zum Ziel, die Entstehung von natürlichen Wirkstoffen zu verstehen, sondern auch, das natürliche Biosynthesepotenzial zu nutzen, um neue Substanzen darzustellen. Bei den hierzu angewandten biokombinatorischen Techniken stehen Biosynthesewege pharmakologisch relevanter Naturstoffe im Mittelpunkt. Unterstützt wurden diese Projekte von zwei EMBO- und Humboldt-Stipendiaten aus Finnland und Ungarn.

Mikrobielle Pathogenitätsfaktoren stellen einen weiteren Forschungsschwerpunkt der Abteilung Biomolekulare Chemie dar. Besonders intensiv werden bakterielle Endosymbionten aus Pilzen untersucht, die zusammen mit ihren Wirten eine phytopathogene Allianz bilden. Die Evolution derartiger Mehrkomponentensysteme und ihre biomolekularen Interaktionen sind Themen stark vernetzter Gemeinschaftprojekte der Arbeitsgruppen am

biomolecular interactions are the subject of research networks comprising work groups at the HKI, the Friedrich Schiller University and the Max Planck Institute for Chemical Ecology, Jena.

In addition to the basic research projects, the Dept. Biostructure Chemistry operates the institute's state-of-the-art analytical facilities (NMR, MS-Techniques, MALDI-TOF) and supports other HKI departments with metabolome and proteome analyses.

The work groups comprise a multidisciplinary team with a broad range of expertises (chemistry, biochemistry, pharmacology). During the past two years (2004 – 2005) about six diploma and three Ph.D. theses were finished in the department, as well as a habilitation thesis (C.H.), which was awarded the first 'physics and life science' prize of the Beutenberg Campus. The first dissertation that emerged from the Junior Research Group (Dr. He Jing, 2005) was honoured by the Chinese government as one of the best Ph.D. theses abroad. Other important awards include the 'DECHEMA Award for Natural Product Research' 2005 (to C.H.) and the Thuringian Science Award 2005 (to C.H. and Laila Partida) for their work on toxin-producing bacterial endosymbionts in fungi, which has been published in *'Nature'* recently.

HKI, der Friedrich-Schiller-Universität und des Max-Planck-Instituts für Chemische Ökologie, Jena.
Zusätzlich zu der anwendungsorientierten Grundlagenforschung betreibt die Abteilung Biostrukturchemie die Hochleistungsanalytik des Instituts (NMR, MS-Techniken, MALDI-TOF) und unterstützt die anderen Arbeitsgruppen in Metabolom- und Proteomanalysen.

Die Abtelung umfasst ein multidisziplinäres Team vielseitiger Expertise (Chemie, Biochemie, Pharmakologie). In den Jahren 2004 – 2005 wurden in den Abteilungen 6 Diplom- und 3 Promotionsarbeiten abgeschlossen, sowie eine Habilitationsarbeit (C.H.) angefertigt, für die der erste Wissenschaftspreis „Physik und Lebenswissenschaften" des Beutenberg Campus verliehen wurde. Die erste Promotionsarbeit aus der Nachwuchsgruppe (Dr. He Jing) wurde von der Chinesischen Regierung als eine der besten Dissertationen im Ausland ausgezeichnet. Weitere wichtige Preise sind der DECHEMA Nachwuchswissenschaftlerpreis für Naturstoff-Forschung 2005 (C.H.) und der Thüringer Wissenschaftspreis 2005 (für C.H. und Laila Partida), für die in *„Nature"* veröffentlichen Arbeiten zu Toxin-bildenden bakteriellen Endosymbionten in Pilzen.

Scientific Projects

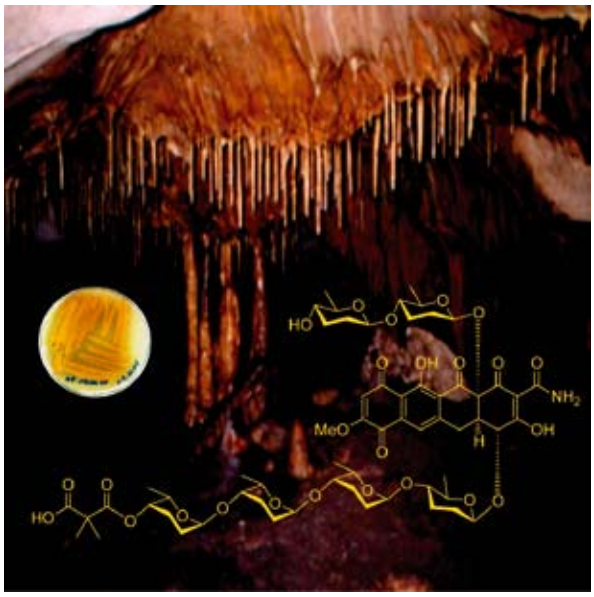
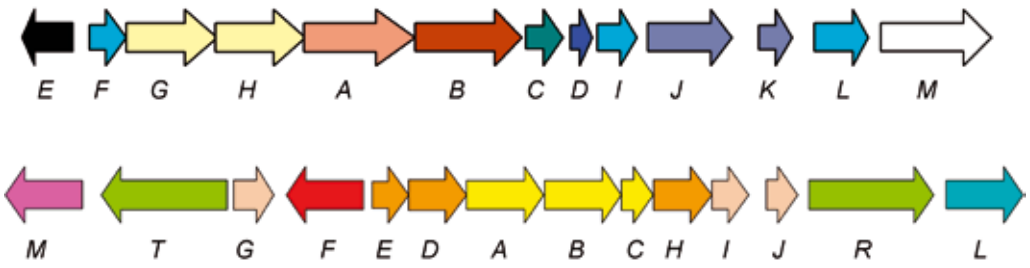


Figure 1
Origin of the cervimycin producer (left), the Grotta dei Cervi, Italy, and chemical structure of the antibiotic

1 Isolation and Structural Elucidation of Bioactive Natural Products

Novel Antibiotics from a Cave Bacterium

Gram-positive bacteria, such as *Staphylococci*, are the most common cause of severe nosocomial infections. The resistance of these pathogens to many commonly used antibiotics, such as penicillins and tetracyclines, has increased dramatically. Until recently, the glycopeptide antibiotic vancomycin has been considered as the last resort against serious infections caused by multi-drug-resistant Gram-positive bacteria. The occurrence of life-threatening infections and the lack of new powerful antibiotics to combat such pathogens cause serious concerns in the medical community. In a program for searching new anti-infective agents we have studied the biosynthetic capabilities of rare microorganisms and inhabitants of unusual habitats with promising potential for metabolite production, such as ancient caves (in collaboration with Dr. I. Groth, MNF, funded by EU). The *Grotta dei Cervi*, Italy, which is not accessible to the public, harbors a rich microbial flora that remained unaffected since ancient times. We found that a bacterial strain isolated from a rock wall, which was covered with 5000 year old neolithical wall paintings in red ochre or black bat dung, produces a novel complex of antibiotics, named the cervimycins. A large number of very potent antibacterial polyketide glycosides was isolated from scaled-up fermentations (collaboration with the pilot plant) and their structures were fully elucidated by extensive NMR studies and degradation experiments. The cervimycins were tested for their bioactivity profile (collaboration with Dept. of Immunology), which revealed that they are highly active even against multi-resistant bacterial pathogens, and are also defeating vancomycin resistance. On the basis of an institute-wide basic research network this promising candidate is further developed and its target elucidated. (Figure 1)



A

B

Figure 2
A Organization of two cloned and sequenced biosynthesis gene clusters;
B production of novel bioactive metabolites by genetically engineered bacteria

2 Functional Genomics of Microbial Secondary Metabolism

Analysis of Genes Encoding Polyketide Antibiotic Biosynthesis Pathways

Polyketides constitute one of the most important and most structurally diverse group of natural products that possess broad ranges of pharmacological properties and, together with their semi-synthetic derivatives, command a vital role in human and veterinary medicine. They derive their enormous diversity in structure through a number of programmed events that are dictated by polyketide synthases (PKS) in a fashion reminiscent of fatty acid biosynthesis. The Bioorganic Synthesis group and the Molecular Antibiotics Biosynthesis group jointly take part in the BMBF-funded project "Secondary Metabolite Biosynthesis Genes from *Streptomyces*: Functional Analysis and Usage for Synthesis of Modified Polyketides", which is part of the BMBF-funded GenoMik competence network. The project aims at a detailed molecular analysis of secondary metabolites from Streptomycetes that are derived from mixed polyketide biosynthetic pathways. Cloning, sequencing and heterologous expression of biosynthetic gene clusters will give information on the biosynthesis of selected hybrid metabolites, the organization of their biosynthetic genes and their functions. Numerous biosynthetic gene clusters from Actinomycetes have been cloned and sequenced in our workgroup, which set the basis for generating novel biosynthetic pathways by the targeted recombination of genes in the sense of 'white biotechnology'. (Figure 2)

Eukaryotic Polyketides in Surrogate Hosts

Many eukaryotic organisms contain genes encoding the biosynthesis of complex compounds often endowed with pharmaceutically useful biological properties. Traditional

methods for exploiting these compounds have included harvest and extraction of organisms, or fermentations. However, for many organisms these methods are ineffective. In addition, the biosynthetic pathways and tailoring steps are often complementary. Thus, co-expression of biosynthetic genes may generate new metabolic diversity. The junior research group Bioorganic Synthesis takes part in an EU-funded project "EUKETIDES-Eukaryotic Polyketides in Surrogate Hosts". The aim of this project is to gain insight into the yet little understood biosynthesis of polyketides in fungi, lichen and algae. The EUKETIDES consortium has obtained a plethora of genes directly from a variety of unculturable eukaryotes using new genetic tools. A range of fungal polyketide synthase genes have been identified and characterized, which have been employed for heterologous expression in various hosts. In addition, silent biosynthesis gene clusters have been identified through reverse genetics and genome sequences. In a joint effort involving three work groups at the HKI, strategies for the systematic exploitation of these genes have been developed.

3 Dissecting and Engineering Multi-component PKS Systems

Exploring Polyketide Cyclization Beyond Linear and Angucyclic Patterns

Polyphenolic natural products formed by multi-component polyketide synthases (type II PKS) comprise an important and structurally diverse group of bacterial secondary metabolites. Many of these compounds or their semisynthetic derivatives have emerged as clinically useful drugs or are promising drug candidates, e.g. tetracycline or doxorubicin. So-called cyclases (CYC), which function "chaperone-like", play a pivotal role in aromatic polyketide biosynthesis, as they help directing nascent polyketide intermediates into particular reaction channels. In the presence of these enzymes, spontaneous aldol chemistry is efficiently suppressed. Considering the vast number of bacterial aromatic polyketides, it is remarkable that virtually all polyphenols are formed by a U-shape polyketide folding and thus can be grouped into linear and angular polyketides. The cumulated ("discoid") ring system of resistomycin is a clear exception. Resistomycin exhibits a variety of pharmacologically relevant properties, e.g. inhibition of HIV-1 protease, as well as RNA and DNA polymerase, and activity against Gram-positive bacteria and mycobacteria. In the course of a DFG-funded basic research project we have successfully identified the resistomycin biosynthesis gene cluster by heterologously expressing an entire cosmid library in a heterologous *Streptomyces* host and screening for the fluorescence of the metabolite produced. The functional analysis of the entire gene cluster by targeted deletion, heterologous expression and structural elucidation of novel metabolites we gained the first insight into the molecular basis of a unique mode of cyclization. Biocombinatorial approaches using unusual cyclase and PKS tailoring genes also set the basis for engineering aromatic polyketides. (Figure 3)

Oxidative Rearrangement of a Linear Polyketide Leads to a Potent Antitumor Agent

The polyketide glycoside chartreusin *(cha)* produced by *S. chartreusis* is a very potent antitumoral polyketide glycoside. Its development as drug, however, has been hampered by its unfavorable pharmacokinetics. The polyketide pharmacophore features a unique pentacyclic bis-lactone structure, which results from a puzzling biosynthetic pathway. In collaboration with Combinature Biopharm, Berlin, we have identified the chartreusin *(cha)* biosynthesis gene cluster in the genome of *S. chartreusis* HKI-249. The cha cluster has been fully sequenced and its identity was confirmed by heterologous expression.

Analysis of the cha gene cluster and isolation of resomycin C from a rationally designed mutant proved that chartarin is derived from an anthracyclic progenitor by an unprecedented oxidative rearrangement sequence. A revised biosynthetic model rationalizing the irregular acetate labeling pattern of chartreusin has been presented. Considering that only relatively few polyketide ring systems are realized in Nature, the chartreusin pathway represents

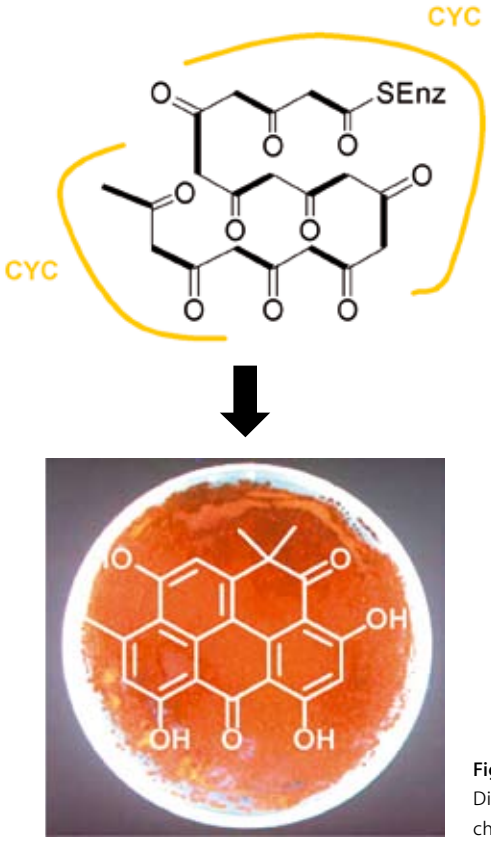


Figure 3
Directed folding of a polyketide chain into a 'discoid' ring system

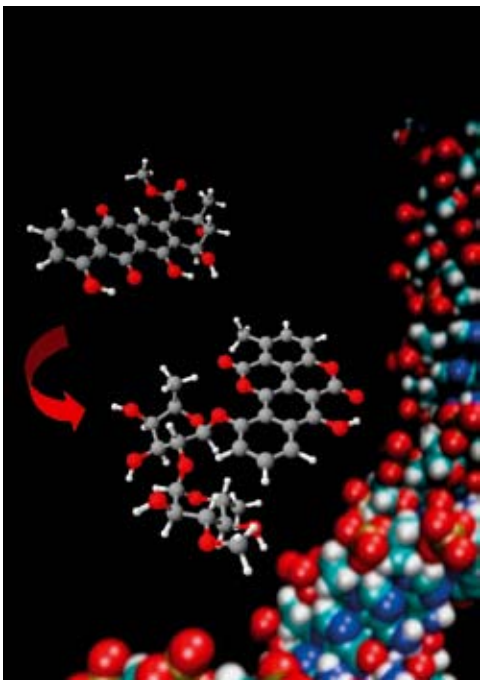


Figure 4
A linear polyphenol is transformed into the potent antitumor agent chartreusin, which intercalates into DNA

another important example of the elaboration of unusual cyclisation patterns. Thus, the cha tailoring enzymes, including novel ring-cleaving oxidoreductases and a putative 2-glycosyl transferase, are not only intriguing from a mechanistic point of view, but are also promising candidates for combinatorial biosynthetic approaches. (Figure 4)

4 Enzymology and Mechanisms of Natural Product Biosynthesis

Biochemical Investigation of Mechanistically Intriguing Oxygenases: a Nitro-forming N-Oxygenase from *Streptomyces thioluteus*

Aromatic nitro groups are relatively rare, albeit widespread structural elements of natural products, most of which are endowed with important biological properties. Surprisingly, to date the biosynthesis of aromatic nitro groups has not been elucidated. We recently cloned an N-oxygenase gene *aurF* from the gene cluster encoding aureothin biosynthesis in *Streptomyces thioluteus*. Inactivation, complementation and expression studies revealed that AurF plays a crucial role in the formation of the novel polyketide synthase starter unit *p*-nitrobenzoate (PNBA) from *p*-aminobenzoate (PABA), and ruled out an involvement of the P_{450} monooxygenase AurH in N-oxygenation. AurF is only the second known N-oxygenase involved in the biosynthesis of a nitro group containing metabolite, besides PrnD from the pyrrolnitrin pathway in *Pseudomonas pyrrocinia*. While PrnD shows a clear Rieske motif, strikingly, AurF represents a novel type of oxygenases, as it does not show any common conserved motifs of known oxidoreductases (e.g. for cofactor binding). Many speculations have been made on the biosynthesis of nitro compounds from amines, yet no hard evidence has been given. By the extraction-free at-line detection of *p*-hydroxylaminobenzoate (PHABA), and by successful transformation of synthetic PHABA

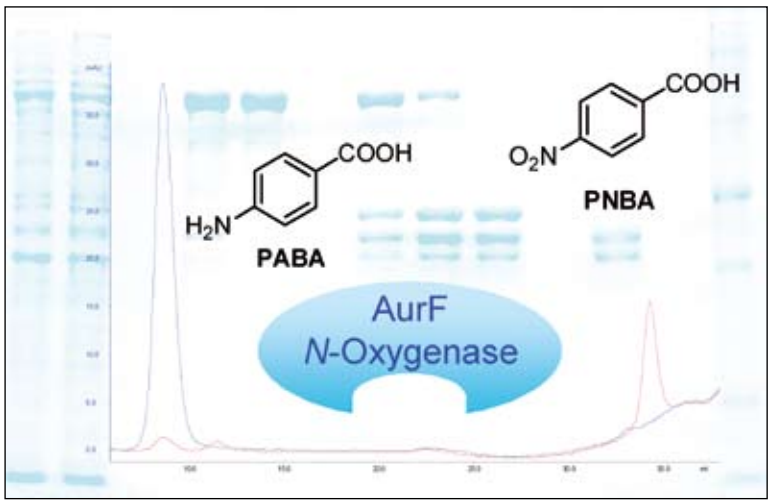


Figure 5
Discovery and characterization of a novel nitro group – forming enzyme. Experimental work was carried out by Robert Winkler within a DFG-financed research project.

to PNBA by heterologously produced AurF we provided the first direct evidence for a stepwise oxidation of aromatic amines to nitro groups via *p*-hydroxylamine as a key intermediate. Our results clearly demonstrated that AurF is a monooxygenase, which not only catalyzes the formation of a hydroxylamine, but also the subsequent oxidation to the aromatic nitro group, presumably by employing the same catalytic mechanism. (Figure 5)

5 Merging the Potential of Biosynthesis and Synthetic Chemistry

Semi-Synthesis of Novel Antimicrobials

The dramatic increase of pathogen resistance and the lack of potent therapeutics e.g. against fungal or nosocomial infections urges the rapid development of novel antimicrobial drugs. 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. Important examples are represented by the erythromycin derivatives dirithromycin, roxithromycin, and azithromycin, which are in clinical use. A second generation of erythromycins, e.g. for treatment of respiratory tract infections, as well as tylosin derivatives, which are used in veterinary medicine have emerged from various synthetic approaches. Yet variants of 16-membered macrolide antibiotics are less explored. With access to bulk quantities of the turimycin complex produced by *Streptomyces hygroscopicus* we are currently exploring (bio)transformations giving rise to derivatives with potentially antibiotic, antiinflammatory and immunomodulating activities. In the past years over 50 variants of macrolides have been synthesized in our work group by modern synthetic methods. These new compounds are currently evaluated by our in-house screening fa-

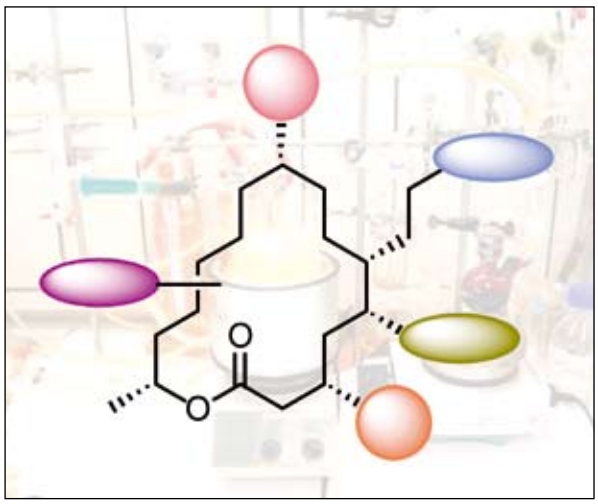


Figure 6
Using macrolides as scaffolds for the semi-synthesis of novel antimicrobials

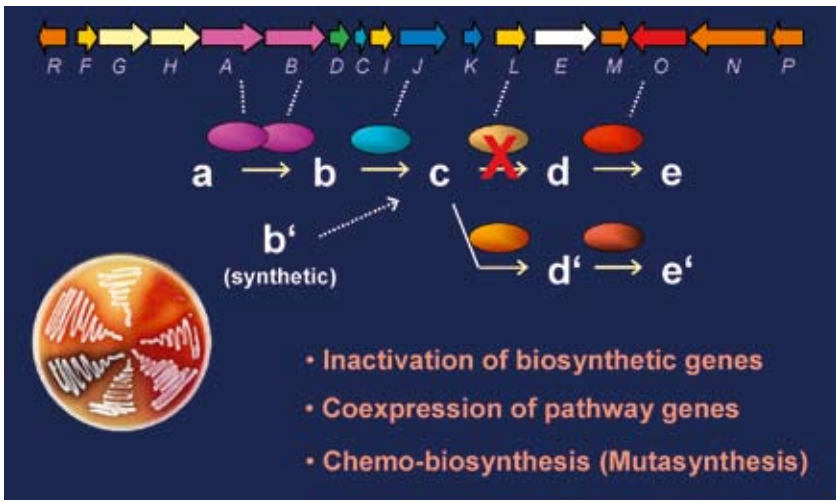


Figure 7
Merging the potential of biosynthesis and synthetic chemistry

cilities and by structural biology (collaboration with Dr. Frank Schluenzen, MPI Hamburg). (Figure 6)

New Strategies Based on Molecular Biology

A viable approach to generate natural product analogues that are not accessible through pathway engineering is mutasynthesis. By means of this technique the natural biosynthetic pathway is suppressed, which makes it possible to "force-feed" non-natural biosynthetic building blocks to the manipulated biosynthetic machinery.

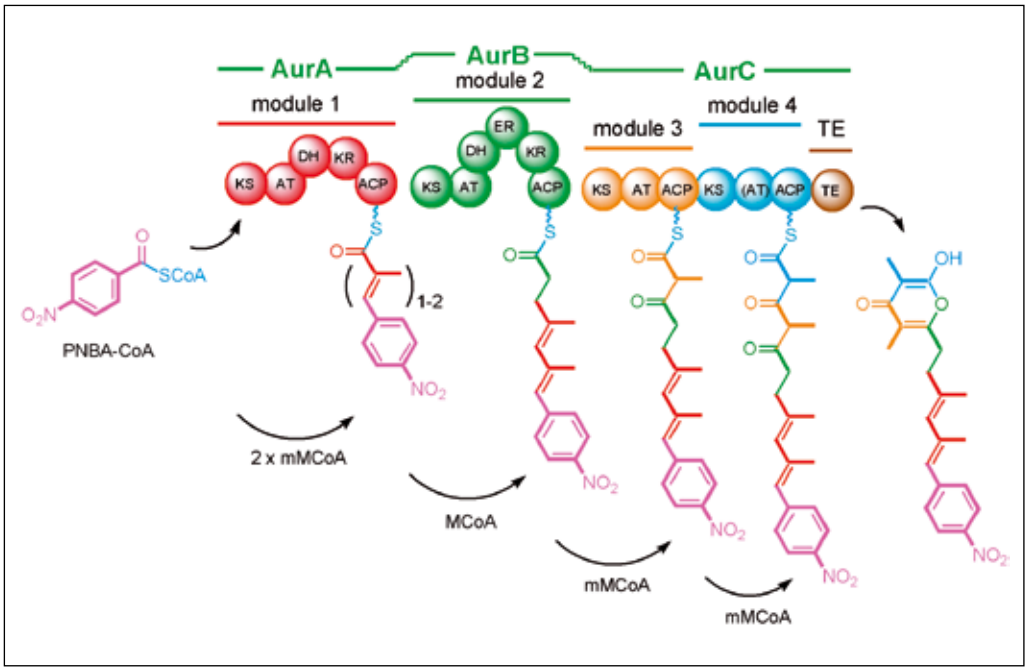
We have successfully explored the potential of the iterative aureothin PKS for precursor-directed biosynthesis. For this purpose, suitable mutants were engineered, which also proved the biosynthetic model of starter unit (PNBA) biosynthesis via PABA involving a specific PABA synthase. For complementation of the mutants, a number of PNBA surrogates were tested as free acids, as well as the corresponding synthetic N-acetyl cysteamine thioesters. The iterative *aur* polyketide synthase allowing p-cyano benzoate besides PNBA to be further processed. The resulting nitrile analog of aureothin, which was fully characterized, shows significantly enhanced cytostatic effects against HeLa and K-562 tumor cells compared to the native compound at a range of 50 µg/ml to 10 ng/ml. This result clearly underlines the power of mutasynthesis towards polyketide derivatives with improved properties. (Figure 7)

6 Evolution of Metabolic Diversity

Non-colinear Polyketide Biosynthesis – A Model System for the Evolution of Modular Polyketide Synthases

Complex polyketides, such as macrolides and polyenes are usually biosynthesized by modular polyketide synthases (PKS), which consti-

Figure 8
Molecular processing line in the biosynthesis of a complex polyketide



tute highly evolved molecular assembly lines. The polyketide backbone is assembled and processed while tethered to the modular thiotemplate. In contrast to iterative PKS systems the number and architecture of the PKS modules usually mirrors the number of elongation cycles and the degree of reduction. However, there are a few exceptions of this 'principle of co-linearity'. By reverse genetics, we have localized a 27 kb gene cluster encoding the biosynthesis of aureothin, an antitumoral and antifungal metabolite from the soil bacterium *Streptomyces thioluteus*. Detailed sequence analyses and heterologous expression of the *aur* gene set revealed that four modules are sufficient for catalyzing five rounds of elongation and processing. The domain architecture of the PKS modules as well as mutagenesis and gene fusion experiments unequivocally revealed that the first module is used twice. The aureothin synthase represents one of the first reported examples in which a modular PKS system breaches with the principle of co-linearity. Comparative analyses with the more recently cloned and sequenced neoaureothin biosynthesis gene cluster have shed more light on the programming and evolution of modular PKS. These unique PKS systems serve as model system for the evolution of microbial polyketide synthases and are investigated in collaboration with Prof. Elke Dittmann, Berlin, and Prof. Rolf Müller, Saarbrücken within the DFG Priority Programme (SPP 1152) "Evolution of Metabolic Diversity" (Figure 8).

7 Unveiling the Molecular Basis of Microbial Virulence

Discovery of Toxin-Producing Bacterial Endosymbionts of the Rice Pathogenic Fungus *Rhizopus microsporus*

Pathogenic fungi generally exert their destructive effects through pathogenicity factors. An important example is the macrocyclic polyketide rhizoxin, the causative agent of

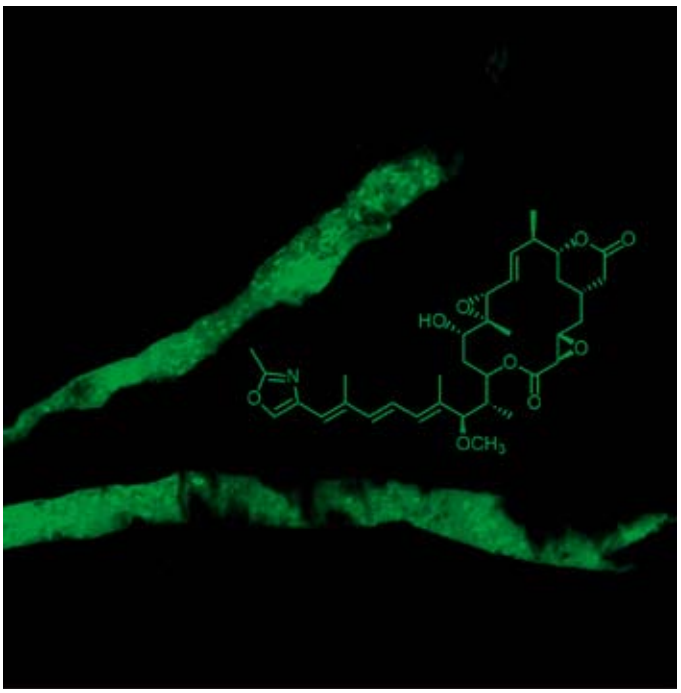


Figure 9
Toxin-producing bacterial endosymbionts (*Burkholderia sp.*) in the mycelium of the rice pathogenic fungus *Rhizopus microsporus* and structure of the antimitotic rhizoxin.

rice seedling blight, from the fungus *Rhizopus microsporus*. The plant disease is typically initiated by an abnormal swelling of the seedling roots caused by rhizoxin without any sign of infection by the pathogen. The phytotoxin efficiently binds to rice ß-tubulin, which results in inhibition of mitosis and cell cycle arrest. Owing to its remarkably strong antimitotic activity in most eukaryotic cells, including various human cancer cell lines, rhizoxin has attracted considerable interest as a potential antitumour drug. By a series of experiments we could unequivocally demonstrate that rhizoxin is not biosynthesized by the fungus, as previously assumed, but by endosymbiotic, that is, intracellular living, bacteria of the genus *Burkholderia*. We were able to cure the fungus from the endosymbionts, yielding a rhizoxin-negative strain proved by cultivation of the symbionts in pure culture that the bacteria are the true producers of the toxin. The association of *Rhizopus* and *Burkholderia* sp. is the first example of a bacterial–fungal symbiosis with a clear metabolic and ecological function. The fungus hosts a bacterial population for the production of the causative agent of rice seedling blight. Rhizoxin, which inhibits mitosis in rice plant cells, efficiently weakens or even kills the plant, and both host and symbiont benefit from nutrients from decaying plant material. This ecologically and medicinally relevant system raises questions about the acquisition and transmission of the symbiont, as well as the evolution of reciprocal adaptation – in particular, resistance towards rhizoxin. In collaboration with Prof. J. Wöstemeyer, FSU Jena we are currently studying this remarkably complex system that extends the fungus–plant interaction to a third bacterial key player. In addition we were able to isolate and characterize novel rhizoxin derivatives from an up-scaled fermentation of the cultured symbionts, which rank among the most potent antimitotic agents known to date. (Figure 9)

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„Kombinatorische Biosynthese von aromatischen Polyketiden durch ein Multiplasmid-Expressions-System" (He3469/1-1, He3469/1-2)
Christian Hertweck

Deutsche Forschungsgemeinschaft
„Die stotternde Aureothin-Synthase als Modellsystem für die Evolution metabolischer Diversität in Streptomyceten" (He3469/2-1)
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Bundesministerium für Bildung und Forschung
Kompetenznetzwerk „Genomforschung an Bakterien für den Umweltschutz, die Landwirtschaft und die Biotechnologie"
Teilvorhaben: „Analyse der Biosynthese gemischter Sekundärmetabolite in Streptomyceten" (0313105)
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Teilvorhaben: „Genetik der Biosynthese gemischter Sekundär-Metabolite in Streptomyceten" (031U213D)
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European Union
5. Rahmenprogramm der EU, Schwerpunktthema: "Quality of Life and Management of Living Resources"
"Eukaryotic polyketides in surrogate hosts" (QLK3-CT-2002-01940)
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German-Israeli Foundation
"Focusing on Hormone-Independent Breast Cancer" (I-847-221/20049)
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(HKI authors in bold)

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Department of Molecular and Applied Microbiology*

Head since February 2005:
Prof. Dr. Axel A. Brakhage

*Since 2005 the department is entitled "Department of Molecular and Applied Microbiology" and headed by Axel Brakhage. It is based on the former Department Applied Microbiology

INTRODUCTION | EINLEITUNG

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).

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*

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

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 100 % liegt. Die wichtigsten Erreger systemischer Pilzinfektionen sind Hefen der Gattung *Candida*, insbesondere *Candida albicans*, sowie Hyphenpilze der Gattung *Aspergillus*, darunter hauptsächlich *A. fumigatus*.

can be divided into three categories: allergic reactions 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 %. (Sole et al., 2005; *Clin Microbiol Infect* 11, 359-365)

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

Aspergillus fumigatus ist heute das wichtigste über die Luft verbreitete pilzliche 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 Iden-

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 analyse 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 physiology/biochemistry, signal transduction, improvement of genetic techniques, genomics, proteomics, transcriptomics, glycoconjugates, 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.

Regulation of Fungal Secondary Metabolism Genes

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

tifikation von Virulenzdeterminanten gestatten. Die inzwischen erfolgte Sequenzierung des kompletten Genoms von *A. fumigatus* erlaubt zudem in exzellenter Weise, pilzliche Infektionsmechanismen in ihrer gesamten Breite zu studieren.

Die Mitarbeiter der Abteilung Molekulare und Angewandte Mikrobiologie beschäftigen sich mit allen Aspekten der Pathobiologie von *Aspergillus fumigatus*. Die Forschungsarbeiten beinhalten die Gebiete Physiologie/Biochemie, Signaltransduktion, 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 Virulenzdeterminate 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.

Regulation pilzlicher Sekundärmetabolitgene

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,

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 quesions as under which physiological conditions are gene clusters expressed, what kind of regulatory 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. (In addition, new producers of secondary metabolites have been isolated from unusual habitats.)

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.

unter welchen physiologischen Bedingungen bestimmte Gencluster exprimiert werden und welche regulatorischen Gene daran beteiligt sind. Genaue Kenntnisse der molekularen 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.

Weiterhin werden bisher unbekannte Mikroorganismen aus ungewöhnlichen Habitaten isoliert, die neue, interessante Wirkstoffe produzieren

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.

Scientific Projects

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

Group Leader:
Olaf Kniemeyer, Axel A. Brakhage

Analysis of the Fungal Proteome of *A. fumigatus* and *A. nidulans*

With the completion of several fungal genomes, it has become possible to study protein regulation on a global scale. The genome of *A. fumigatus* has been fully sequenced (Niermann et al. 2005) and the annotation has been completed. The genome consists of eight chromosomes with a total size of 29.4 Mb, of which 9,926 protein-encoding sequences were identified. The best-established separation techniques for proteins are still based on 2D-gel electrophoresis, which allows separating proteins by charge and size. We are currently working on the proteome of the human-pathogenic fungus *A. fumigatus* to obtain a comprehensive overview about the proteins present during environmental changes, stress and infection. This approach can help to identify regulatory networks and proteins important for virulence. Furthermore, we have been studying the proteome of *A. nidulans*, whose genome is also publicly available. Our special interest is the identification of target proteins of transcription factors that are involved in the regulation of secondary metabolites such as penicillin. There are ongoing collaborations with the Departments of Biomolecular Chemistry (Christian Hertweck), the Pilot Plant for Natural Products (Uwe Horn) and Infection Biology (Peter Zipfel).

Optimisation of a 2-D Gel Electrophoresis Protocol for the Human-Pathogenic Fungus *A. fumigatus*

Preparation of protein samples for 2-D gel electrophoresis is a critical step and essential for reproducible results. In many cases, protein precipitation methods are applied to separate samples from contaminating compounds such as salts, nucleic acids, lipids, which would otherwise interfere with the 2D-gel electrophoresis. In particular, the presence of the robust cell wall of filamentous fungi causes problems during sample preparation for 2D-gel electrophoresis of fungal proteins. We compared several protein precipitation methods including a commercial kit. All methods were based on the precipitation of proteins by the application of TCA and/or acetone. A TCA/acetone-precipitation method and a commercial kit gave the best result. Furthermore, we optimised the composition of the lysis buffer (sample preparation solution). In order to achieve a well-focused first dimension separation, proteins must be completely solubilised. For this reason the lysis buffer contains denaturants, in general urea, and non-ionic or zwitterionic detergents. A combination of the two sulfobetaine detergents CHAPS and Zwittergent 3-10 resulted in gels with the highest number of detectable spots. Our optimised protocol resolved more than 1000 proteins on a large format gel with a pH-range from 3-11. By comparison of the protein pattern of *A. fumigatus* grown on glucose versus ethanol the method was verified. In saprophytic filamentous fungi, the expression of catabolic pathways involved in the utilisation of carbon compounds strongly depends on available nutrients in the environment. Energetically favourable carbon sources such as glucose are used preferentially compared to less readily metabolisable carbon sources such as ethanol. During growth on ethanol enzymes involved in the conversion of ethanol to glucose (gluconeogenesis), in the oxidation of ethanol, as well as enzymes involved in the replenishment-reactions of the glyoxylate cycle were up-regulated. During growth on glucose the synthesis of the enzymes for the utilisation of ethanol was reduced.

In more detail, all key enzymes of gluconeogenesis, especially PEP carboxykinase and fructose-1,6-bisphosphatase were up-regulated during growth of *A. fumigatus* on ethanol. In addition, malic enzyme showed a higher abundance during growth on ethanol. As expected, the ethanol utilisation enzymes alcohol dehy-

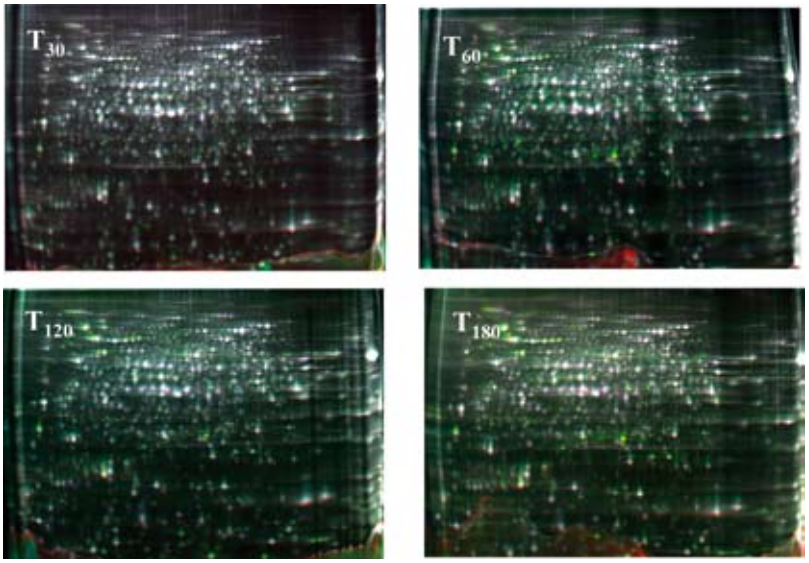


Figure 1
DIGE gels (12.5% polyacrylamide, pH range from 3-11 NL) showing the heat shock response of *A. fumigatus* upon a temperature shift from 30 °C to 48 °C. 50 µg of a protein sample taken at t=0 were labelled with Cy3, 50 µg of protein sample taken after 30, 60, 120 or 180 min were labelled with Cy5. The internal standard was labelled with Cy2.

drogenase and aldehyde dehydogenase were strongly up-regulated in *A. fumigatus* during growth on ethanol. The key enzyme of the glyoxylate cycle isocitrate lyase, was the most up-regulated enzyme with a 415-fold increase. In summary, 35 spots showed a significantly different regulation during growth on ethanol compared to glucose.

Heat Shock Response of *A. fumigatus*

A. fumigatus possesses some characteristics, which allows the fungus to survive in the immunocompromised host. During the infection process *A. fumigatus* has to cope with dramatic changes of environmental conditions. An interesting question is whether *A. fumigatus* possesses a higher stress resistance and better adaptation mechanisms compared to other filamentous fungi. The ability of *A. fumigatus* to grow above 40 °C has been obviously recognized as an essential trait associated to growth in mammals. The reason for this high thermotolerance (growth of *A. fumigatus* between 12 – 56 °C) is unknown. Therefore, temperature shift experiments were carried out and growth at 30 °C and 48 °C was compared at the proteome level using the DIGE-approach (2-D Fluorescence Difference Gel Electrophoresis). 2-D DIGE is a method that labels proteins samples prior to 2-D electrophoresis by forming a peptide linkage between the fluorophore (Cy2, Cy3 and Cy5) and lysine residues within the protein. With this technique it is possible to separate up to three different samples within the same 2-D gel. In general, an internal stan-dard prepared by mixing equal amounts of the two different samples of an experiment is produced to aid spot matching of spots across all gels within one experiment. (Figure 1)

During the temperature shift from 30 °C to 48 °C samples were taken at the time points t=0, t=30 min, t=60 min, t=120 min and t=180 min. The DIGE gels were analysed with the De-

Cyder software 5.0 (Amersham Biosciences). Differently synthesised proteins were selected by fold-change values above 2 at least at one time point. Proteins were regarded as significantly regulated with an ANOVA-statistical test p-value below 0.05. Heat shock proteins, proteins related to oxidative stress, cell cycle regulation, signalling, pre-RNA processing and proteins involved in amino acid and ergosterol biosynthesis were differently regulated during a temperature shift from 30°C to 48°C. The heat shock protein HSP 30 was most highly up-regulated showing a 16-fold increase and a hypothetical protein found also in other fungi and bacteria showed a 2.3-fold up-regulation. In conclusion, the *A. fumigatus* heat shock response showed similarities, but also differences to the response reported for baker's yeast. Interestingly, 50% of all significantly regulated proteins (according to ANOVA test) did not show a significant regulation at the transcriptome level. This finding indicates that proteomic and transcriptomic approaches deliver complementary data. A gene regulatory network was reconstructed using an optimised reverse engineering method (see Bioinformatic group).

2 Virulence of *Aspergillus fumigatus*

Group Leader:
Thorsten Heinekamp, Axel A. Brakhage

Identification of *Aspergillus fumigatus* Pathogenicity Determinants and Characterisation of their Regulation

Aspergillus fumigatus is a ubiquitously distributed saprophytic fungus playing an essential role in recycling carbon and nitrogen sources. However, *A. fumigatus* is also an important opportunistic human-pathogenic fungus. Whereas in immunocompetent hosts the fungus can be a major allergen, it can cause life threatening diseases in severely immunocompromised patients. Due to continued proceedings in transplant medicine and therapy of hematological malignancies over the last years the number of patients under sustained immunosuppression strongly increased. Complications during these therapies often occur by opportunistic infection with *A. fumigatus*, causing severe diseases, e.g., invasive aspergillosis with lethality rates of ca. 90 %, making this fungus the most important airbone fungal pathogen.
The virulence group aims at the identification of pathogenicity determinants enabling the fungus to infect the lung and to grow invasively. To understand the communication between host and pathogen we elucidate the signaling pathways that enable this pathogenic fungus to adapt and survive the drastically altered environmental conditions that they encounter upon infection of the host.

We are interested in the cAMP signaling network and its implication in pathogenicity of *A. fumigatus*. Several elements of the cAMP signal transduction pathway were characterised by us. To test whether these components play a role in pathogenicity, virulence of *A. fumigatus* mutant strains was determined in a low-dose murine model for invasive aspergillosis. In this animal model, immunosuppressed mice are infected intranasally with conidia of *A. fumigatus* strains and survival is monitored. Using this infection model, we could verify an involvement in virulence for several genes of the cAMP signaling network. These elements include the adenylate cyclase encoding gene *acyA* and the G protein subunit-encoding gene *gpaB*. GpaB was found to be an upstream stimulator of adenylate cyclase. Deletion strains with mutations in both genes showed reduced conidiation. Whereas the growth rate of the Δ*acyA* mutant was strongly reduced, no reduction of the growth rate was observed for the Δ*gpaB* mutant.

The dihydroxynaphthalene (DHN)-like melanin biosynthesis pathway has been shown to play also an important role in virulence in some pathogenic fungi, including *A. fumigatus*. The genes involved in the DHN-like melanin production in *A. fumigatus* form a cluster, with a polyketide synthase as a key enzyme. Mutants with deletion of the polyketide synthase encoding gene *pksP* produce white conidia and display a strong attenuation of virulence. In contrast, *abr2*, the last gene of

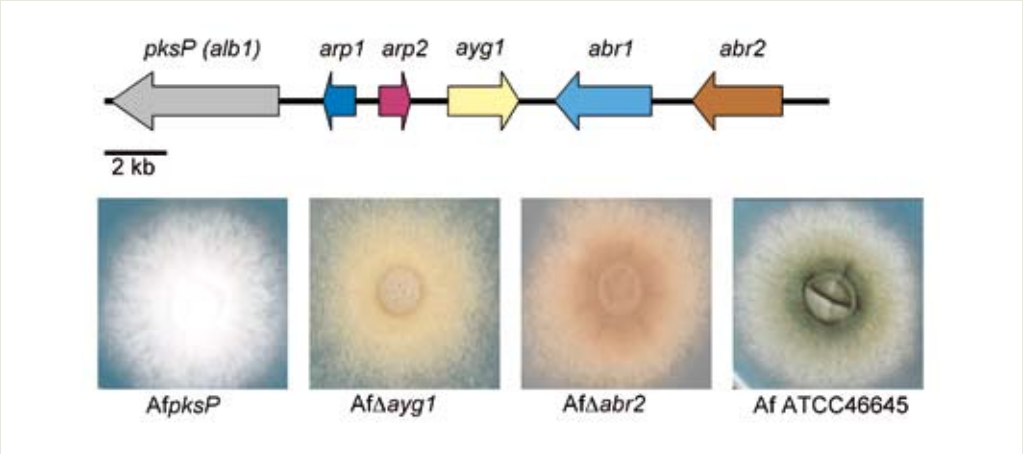


Figure 2
Phenotypical characterisation of different *A. fumigatus* strains, deficient for specific genes of the dihydroxynaphthalene (DHN) melanin biosynthesis gene cluster. Growth and sporulation of *A. fumigatus* strains Δ*ayg1*, *pksP* and Δ*abr2* in comparison to the wild-type strain ATCC46645.

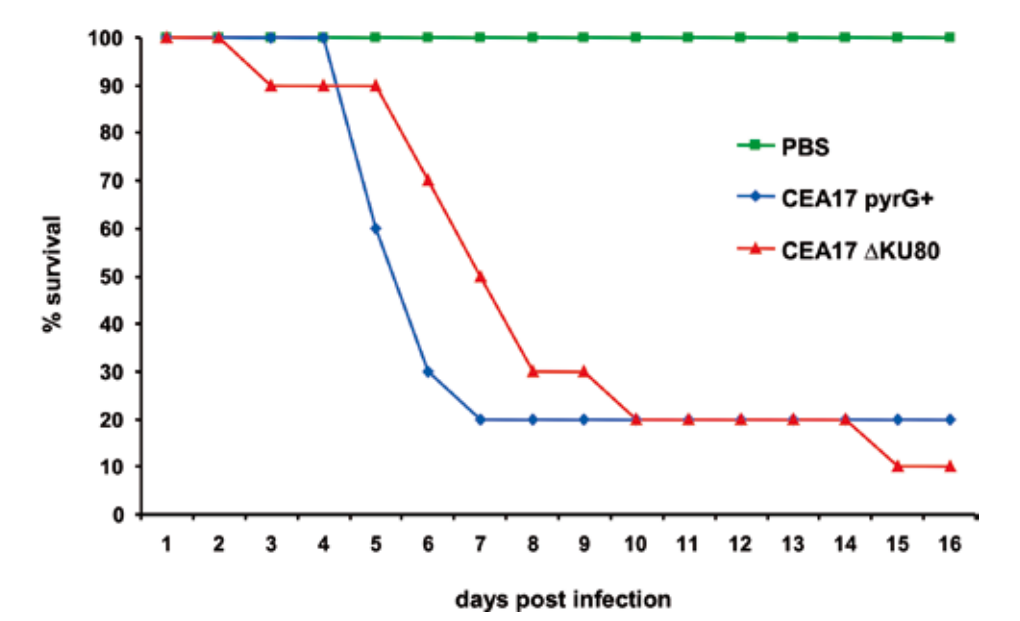
the pigment biosynthesis cluster coding for a laccase, did not contribute to virulence. Therefore, it is conceivable that not melanin by itself, but rather an intermediate of the DHN-like melanin biosynthesis is an important virulence determinant.

Interestingly, the expression of the pathogenicity determinant-encoding gene *pksP* measured as a *pksP*p-*lacZ* gene fusion was reduced in the Δ*gpaB* mutant. Moreover, the killing rates of conidia of *acyA* and *gpaB* deletion strains by human monocyte-derived macrophages were significantly greater than the killing rate of wild-type conidia. These findings suggested that cAMP triggers the defense system of *A. fumigatus* to protect the organism against attack by host immune effector cells.

Some data suggest that other pathways might be cross-talking with the cAMP signal transduction. Possible candidates for such elements are mitogen activated protein kinases (MAPKs). A genome wide analysis indicated that four genes coding for MAPKs are present in *A. fumigatus*, designated *sakA/hogA*, *mpkA*, *mpkB, and mpkC*. Currently, we are analysing in detail the so far uncharacterised MAP kinases MpkA and MpkB by deletion of the corresponding genes.

As a new tool for effective generation of knock-out mutants we employ *A. fumigatus* strains which are defective in non-homologous end-joining DNA repair. In general, transformation of *A. fumigatus* is affected by two independent mechanisms of DNA repair. Integration of foreign DNA is either achieved by KU70/80 dependent non-homologous end-joining DNA repair, resulting in ectopic integration, or by homologous recombination at the desired locus. In *A. fumigatus* ectopic integrations are favoured. Therefore, targeted knock-out frequency in *A. fumigatus* wild-type strains is only 1 to 5% of all transformants. In contrast, transformation of *A. fumigatus* mutants with a deletion in the *akuB* gene, the fungal homolog to the human *KU80* gene, enhances DNA integration at the homologous locus to

Figure 3
Virulence of *A. fumigatus* strains in a murine low-dose model of invasive aspergillosis. Survival of mice infected intranasally with conidia of *A. fumigatus* CEA17pyrG+ (wild type) and KU80Δ, respectively, was monitored for 16 days. Mice were tested in cohorts of 10 animals. A control group (inhalation of PBS) remained uninfected to monitor the influence of the immunosuppression procedure on vitality. The results indicate that the *akuB*^{KU80} mutation is not causing any loss of virulence in the KU80Δ strain compared to the corresponding wild-type strain.



nearly 100 %. Strains deleted in the *akuB*^{KU80} gene display no differences in morphology and pathogenicity in comparison to the corresponding wild-type strains. (Figure 3)

We make use of this new tool to generate knock-out strains of putative G protein coupled receptors. This aims at the identification of external signals and their corresponding receptors sensing those stimuli which activate downstream signal transduction pathways.
In collaboration with Prof. H. Hof and Dr. C. Kupfahl (University of Heidelberg) we characterised mutants deficient in gliotoxin biosynthesis. Gliotoxin is a secondary metabolite produced by several fungi including *A. fumigatus*. Since gliotoxin exerts immunosuppressive effects *in vitro* and *in vivo* a role as virulence factor in aspergillosis has been discussed for a long time but evidence has not been provided until now. Therefore, we constructed strains of *A. fumigatus* deficient for the non-ribosomal peptide synthetase GliP, the putative key enzyme in the biosynthesis of gliotoxin. The analysis of the strains is under way.

In cooperation with Dr. M. Gunzer from the GBF (Braunschweig) dynamic imaging of *A. fumigatus* phagocytosis using DsRed2 as fluorescence marker was performed. The major immune effector cells responsible for defense against the infection have never been directly compared in their phagocytic capability. Furthermore, the outcome of phagocytosis of *A. fumigatus* has been analyzed mostly with fixed samples. To address these questions, *A. fumigatus* strains were generated, which expressed the red fluorescent protein DsRed2 under control of two differentially active promoters, i.e., the lowly expressed polyketide synthase (*pksP*) and the highly expressed isocitrate lyase (*acuD*) gene promoter. Using these strains, different expression patterns of the genes were observed. Moreover, highly resolved dynamic images of the process of phagocytosis by the major phagocytes over a

time course of up to 15 hours were obtained using the *acuD*p-DsRed2-expressing *A. fumigatus* strain.

3 Transcription Factors and Signal Transduction

Group Leader: Axel A. Brakhage

Regulation of Fungal Secondary Metabolism Genes

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. Up to now, the greatest progress in elucidation of the molecular regulation of the biosynthesis of a fungal secondary metabolite has been made in the penicillin-producer *Aspergillus (Emericella) nidulans*, because this fungus is an ascomycete with a sexual cycle. Hence, classical genetic techniques can be applied to *A. nidulans* which, together with molecular techniques, facilitate a thorough analysis of the genetic regulation of metabolic pathways, including that of the biosynthesis of secondary metabolites.

The focus of our group is the identification and characterisation of regulatory proteins, that are involved in the biosynthesis of secondary metabolites, i.e., to answer questions as under which physiological conditions are gene clusters expressed, what kind of regulatory 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.

The CCAAT-Binding Complex AnCF

All eukaryotes analysed so far encode a heterotrimeric CCAAT binding complex, which is highly conserved from yeast to man. In *Aspergillus nidulans* the respective complex is designated AnCF and composed of the three subunits HapB, HapC and HapE. AnCF was found to bind to certain CCAAT boxes, present in the promoter region of various genes.

We showed that in *A. nidulans*, the two penicillin biosynthesis genes *aatA* and *ipnA* are positively regulated by the AnCF complex. Although the work on *A. nidulans* has led to new insights into the structure and function of this complex, hardly anything is known about the regulation of this transcriptional complex, e.g. by external stimuli. Previous results implied, that CCAAT binding complexes might be regulated by the redox status of the cell. Oxidative as well as antioxidative conditions have been shown to alter the DNA-binding activity of several other transcription factors. Our aim is, to investigate, whether the cellular redox environment might be an important post-transcriptional regulator of AnCF subunit association and its DNA-binding activity. If this is the case, this regulation by the redox status of the cell might be a general mechanism for all CCAAT binding complexes.

HapC and the Thioredoxin System of *Aspergillus nidulans*

Interestingly, only HapC contains three cysteine-residues, which could be targets for reactive oxygen species. *In vitro* analysis indicated that HapC can easily dimerise under oxidative conditions. Electrophoretic mobility shift assays (EMSAs) using all three subunits indicated that the CCAAT-binding activity of the AnCF complex was enhanced when HapC was fully reduced.

In a first attempt, we exchanged the three cysteines of HapC against serine, leading to a mutagenised HapC protein without any redox sensitive thiol groups. Interestingly, this recombinant triple-cysteine mutant of HapC showed reduced affinity to HapE *in vitro*, while the formation of the whole AnCF complex was not affected. This result leads to the conclusion, that not only the redox status of the HapC cysteines is important for the assembly of the AnCF complex, but also at least one cysteine residue itself.

Furthermore, we have been addressing the question whether an intracellular redox system is involved in the redox regulation of HapC. Therefore, the two genes encoding the major thioredoxin of *A. nidulans (trxR)* and its corresponding thioredoxin reductase were cloned and the protein products purified. Thioredoxins are small disulfide-containing redox proteins that have been found in all kingdoms of living organisms. Thioredoxins serve as general protein disulfide oxidoreductases. They interact with a broad range of proteins by a redox mechanism based on reversible oxidation of two cysteine thiol groups to a disulfide. In an NADPH-dependent protein disulfide reduction, thioredoxin reductases catalyse reduction of oxidised thioredoxins by NADPH using FAD as cofactor. Reduced thioredoxins then directly reduce disulfide-bridges in the substrate proteins.

Three independent activity assays based on the consumption of NADPH showed a specific disulfide reducing activity of the thioredoxin system from *A. nidulans*, composed of the two recombinant enzymes thioredoxin A and thioredoxin reductase and NADPH. Further studies will show, whether intra- and/or intermolecular HapC-disulfide-bridges are molecular targets for thioredoxin A from *A. nidulans*.

4 Physiology and Biochemistry of Filamentous Fungi

Group Leader: Matthias Brock

Interaction of Biosynthesis Pathways and Impact on Virulence

Primary metabolism is proposed to be essential during infectious processes of microorganisms to obtain nutrients for growth and maintenance in infected tissues. However, only little is known about the metabolic requirements, which may be strongly dependent on the site of infection.

Aspergillus fumigatus is able to cause the so-called invasive aspergillosis in immunocompromised patients and generally starts with colonisation of lung tissues. Alveolar macrophages and neutrophils as the primary barriers against fungal colonisation are weakened or absent in immunocompromised individuals, which enables the outgrowth of the fungus. During growth through the lung tissue epithelial cells are destroyed, leading to large necrotic parts destructing the lung. Additionally, growing fungi can reach the lymph and blood stream and spread to other organs accompanied with a high mortality of patients.

Due to the fact that *A. fumigatus* is found ubiquitously as a main decomposer of organic material, the fungus possesses a versatile metabolic capability and can rapidly adapt to changing environmental conditions. Therefore, it is one of the main focuses to identify metabolic pathways, which may be required during infection and to elucidate the biochemical properties of selected enzymes. When identified as contributing to virulence these enzymes provide possible targets for antifungal drug development. Promising candidates for important metabolic pathways are the methylcitrate cycle for degradation of propionyl-CoA, the glyoxylate cycle for *de novo* synthesis of oxaloacetate during growth on C2-generating carbon sources and the lysine biosynthesis *via* the alpha-aminoadipate pathway. All three pathways are lacking in humans and therefore might represent suitable targets for antifungal drug development.

The Methylcitrate Cycle

The methylcitrate cycle is essential for growth on propionate and for the removal of toxic propionyl-CoA. This cycle converts propionate by an alpha-oxidation into pyruvate and possesses several specific enzymes. Propionate is activated by propionyl-CoA synthetase to yield propionyl-CoA. Alternatively, the degradation of odd chain fatty acids and the degradation of the amino acids valine, isoleucine and methionine directly lead to propionyl-CoA. A condensing reaction of propionyl-CoA with oxaloacetate *via* a cycle specific methylcitrate synthase leads to methylcitrate as the first specific intermediate of this cycle. Methylcitrate is dehydrated by a methylcitrate dehydratase, which acts in an unusual manner, because it abstracts the proton and the OH-group *in syn*, which is generally not observed in dehydration reactions from malate derivatives. The methyl-*cis*-aconitate formed in

this reaction is rehydrated by an unusual form of the citric acid cycle aconitase, which is active on this substrate by containing a [3Fe-4S] instead of a [4Fe-4S] cluster in the active site. The product methylisocitrate is cleaved by the last cycle-specific enzyme methylisocitrate lyase into succinate and pyruvate. Succinate is regenerated to oxaloacetate, whereas pyruvate can be used for energy metabolism and biomass formation.

Propionate is often used as a preservative (E280 – E283) and added to several pastries, white bread and feed. The addition of propionate has two beneficial effects. On the one hand growth of filamentous fungi is inhibited, when this compound is added to glucose-containing media and additionally, high concentrations of propionate prevent the synthesis of toxic secondary metabolites like ochratoxin A or aflatoxins.

We investigated the methylcitrate cycle in the model organism *Aspergillus nidulans* and the opportunistic human pathogenic fungus *A. fumigatus*. In both organisms a deletion of the methylcitrate synthase led to the inability of the mutants to use propionate as a substrate for growth. Furthermore, the addition of propionate to glucose-containing medium reduced growth much stronger than observed in the wild type. Investigation of this phenotype showed that the deletion mutants accumulated large amounts of propionyl-CoA during co-metabolism of propionyl-CoA generating carbon sources. Propionyl-CoA was shown to act as a competitive inhibitor of the pyruvate dehydrogenase complex with a K_i of 50 μM. Furthermore, the succinyl-CoA synthetase from the citric acid cycle was severely inhibited by propionyl-CoA. These effects can easily explain the increased growth inhibition of the mutant strains. Besides these growth inhibitory effect the polyketide synthesis of deletion mutants was strongly impaired. Polyketides like sterigmatocystin, naphthopyrone, ascoquinone A or dihydroxynaphthalene (DHN)-melanin were no longer formed in the mutants when propionyl-CoA accumulated. Therefore, it was concluded that the efficient removal of propionyl-CoA is a prerequisite for normal growth behaviour and efficient polyketide synthesis. The *A. fumigatus* methylcitrate synthase mutant was also investigated for a possible attenuation in virulence in a murine low dose infection model of invasive aspergillosis. Proteins are supposed to be a major carbon source for growth of *A. fumigatus* during infection. Cleavage of proteins by secreted proteases and peptidases leads to release of free amino acids, which can be taken up and used as nutrients. Among these are valine, isoleucine and methionine which are metabolised and lead to propionyl-CoA as mentioned above. The inability of the mutant to remove this propionyl-CoA leads to accumulation of this compound and reduced growth. Comparison of a wild type and a mutant strain revealed a ten times attenuated virulence of the mutant and additionally, thin sections of infected lungs showed an embanked growth of the mutant, whereas the wild type invasively grew through the tissue. (Figure 4) Therefore, an intact methylcitrate cycle seems to be essential to retain full virulence and proteins indeed seem to serve as a carbon source during infection.

Besides the methylcitrate synthase we will test the attenuation of other methylcitrate cycle mutants in the murine infection model. First analysis of a methylisocitrate lyase mutant from *A. nidulans* showed that such a strain is even more sensitive against addition of propionate by accumulating high amounts of methylisocitrate. This compound strongly inhibits the NADP dependent isocitrate dehydrogenase and therefore also impairs growth and development. Investigations on an *A. fumigatus* mutant will show, whether this is also important under infectious conditions.

The Glyoxylate Cycle

Besides protein degradation lipids may serve as an additional carbon source during infection. It was shown for several pathogenic microorganisms that isocitrate lyase is of importance for maintenance of infection. Experiments with *A. fumigatus* have shown that isocitrate lyase is induced in the presence of C2 generating carbon sources and a deletion strain is unable to grow on fatty acids, acetate or ethanol. Therefore, no alternative enzyme is able to take over the role of isocitrate lyase. Additionally, isocitrate lyase

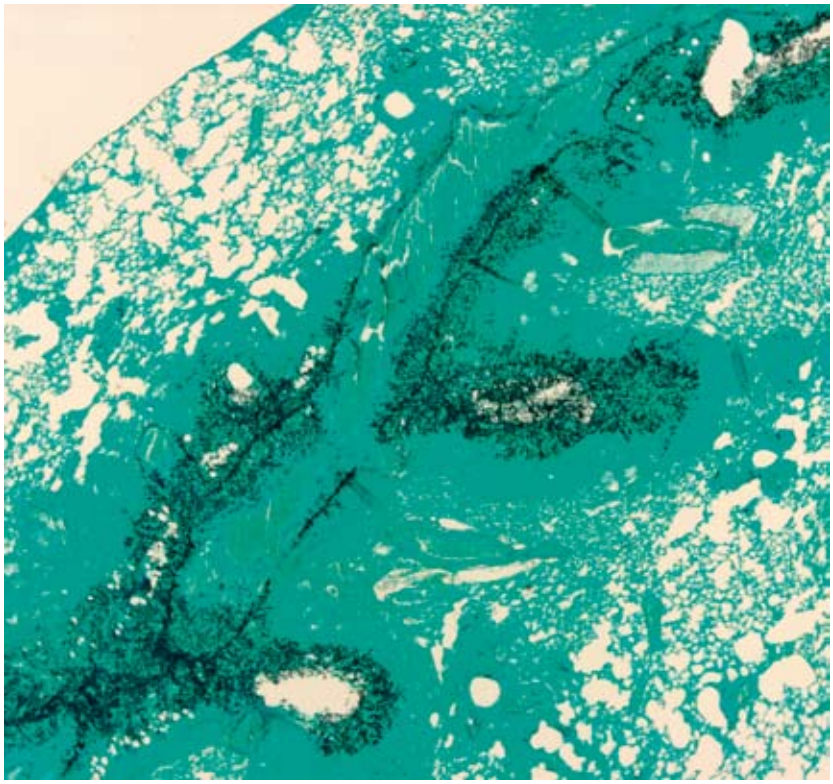


Figure 4
Silver staining of a lung thin section 4 days after infection with an *Aspergillus fumigatus* wild-type strain. The lung tissue appears in blue, whereas mycelium is stained black (20 times magnified; preparation of lung sections in collaboration with J.-P. Latgé, Paris).

is always present in conidia, which implies that the enzyme may be involved in the metabolism of stored nutrient like lipids, which were shown to be present in conidia. Furthermore, as a soil microorganism acetate is an abundant carbon source and the presence of isocitrate lyase in the toolbox of conidia may be important for rapid adaptation. Isocitrate lyase can also be observed, when germination occurs within macrophages implicating that at least some of the available nutrients from macrophages may have a lipid origin. However, when tested in a murine infection model, isocitrate lyase mutants did not display an attenuated virulence. Therefore it is unlikely that *A. fumigatus* depends solely on C2 generating carbon sources during infection. Nevertheless, it cannot be excluded that fatty acids and lipids may serve as an additional carbon source, which may therefore be independent from the glyoxylate cycle. Therefore, further studies will focus on enzymes involved in lipid degradation to study their impact in virulence.

Lysine Biosynthesis

Lysine is synthesised *de novo via* the alpha-aminoadipate pathway. A functional lysine biosynthesis is required for virulence since a deletion of the homoaconitate dehydratase leads to conidia, which are unable to germinate within infected lung tissues. In order to study, whether the enzyme may be a suitable target for antifungals, we aim at the purification of the enzyme. Since homoaconitate dehydratase seems to contain a labile iron sulphur cluster and is additionally only produced in low amounts, purification is severely hampered. To overcome these problems the gene coding for homoaconitase has been expressed under the inducible isocitrate lyase promoter, which significantly increased enzymatic activity in crude extracts. Furthermore, in a cooperation project a monoclonal antibody against a domain of the homoaconitase was produced. This antibody will be coupled to chromatographic materials and used for affinity purification of the homoaconitase from the overproducing

strain. Affinity purified homoaconitase from *A. fumigatus* will be the first purified homoaconitase and studies on the biochemical properties will give new insights in the reactions catalysed by this enzyme.

Metabolomics

Besides investigations on specific pathways, the metabolic capacities of *A. fumigatus* are an additional focus of our investigations. This research involves the identification both of primary and secondary metabolites. For that purpose, mycelium has been grown with different carbon sources. Metabolites have been extracted from both the mycelium and the medium. The mycelium mainly contains primary metabolites, whereas secondary metabolites are generally excreted to the medium. For identification, metabolites are derivatised by incubation in methoxyamine hydrochloride solved in pyridine and trimethylsilylated in MSTFA (N-methyl-N-trimethylsilyl-triflouracetamide). This procedure helps to enhance resolution during gas chromatography, lowers the detection limit and increases thermal stability. After gas chromatography metabolite masses are determined by mass spectrometry and compared to database entries. Unknown masses are further investigated by MS-MS and mass fragments can be analysed by pattern recognition. This investigation will help to identify the metabolic pathways utilised at specific growth conditions.

5 Protein-Protein Interactions

Group Leader: Peter Hortschansky

Functional Analysis of Multi-Component Protein Complexes

Proteins are one of the key players of life. Knowledge about their interactions at the molecular level within protein complexes or with nucleic acids is of essential importance for the understanding of cellular processes and the mechanisms of disease. The first step during characterisation of these interactions *in vivo* is to address the question whether the proteins interact with each other and to what extent *in vitro*. Unfortunately, in *Escherichia coli* many target proteins are not efficiently synthesised in a folded or soluble form as a result of the complexity of folding and the limited solubility. To obtain proteins and peptides for functional and interaction analysis we use our expertise in microbial cultivation, fusion protein engineering, *in vitro* refolding and protein purification. Interaction specificity and affinity is measured by the surface plasmon resonance (SPR) technique (Biacore).

Transcription Factor Complexes of *Aspergillus nidulans*

CCAAT pentanucleotide sequences are present in approximately 30% of the promoter regions of eukaryotic genes. A heterotrimeric complex designated Hap (Heme activator protein) was found to bind to CCAAT sequences in *Saccharomyces cerevisiae*. Since this discovery, this regulatory complex has been found in all eukaryotes which have been analysed. Functional conservation of Hap homologs of *A. nidulans*, humans and *S. cerevisiae* exists. In recent years, work on *A. nidulans* has led to new insights into the structure, regulation and function of the CCAAT-binding complex. For example, AnCF regulates the genes involved in the biosynthesis of penicillin. A common feature of these homologous complexes is that three different subunits are necessary for formation of a DNA binding protein complex, designated AnCF in *A. nidulans*. The general mode of interaction between the subunits in higher and lower eukaryotes appears to be similar. Only the HapB subunit contains a nuclear localisation signal. Therefore, the HapB subunit is the primary cargo for the import machinery, while HapC and HapE are transported to the nucleus only as a heterodimer and in complex with HapB via a piggy back mechanism.

Until now, no detailed information concerning subunit interactions during AnCF CCAAT-binding complex assembly or AnCF DNA-binding affinity is available. Therefore, the analysis of the CCAAT-binding complex leads to new insight in both the regulation of secondary metabolism genes and the function of an important transcription complex present in all eukaryotes. For the first time we were successful in purifying all Hap subunits and the ba-

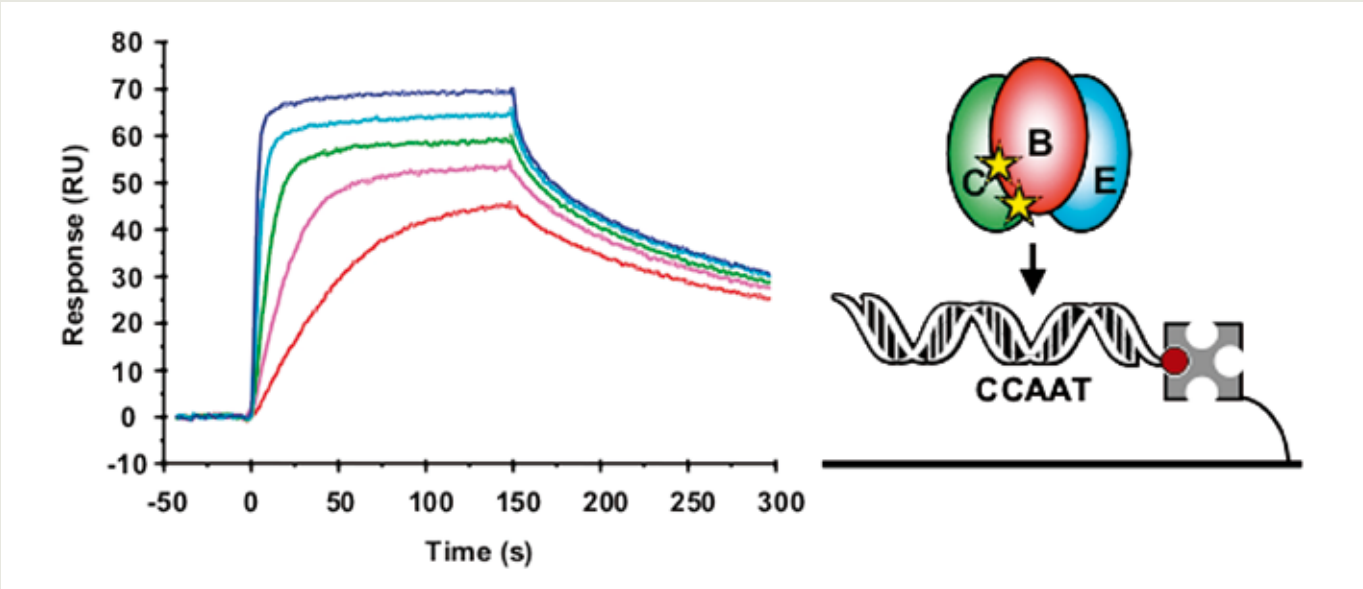


Figure 5
Kinetic analysis of *A. nidulans* AnCF complex binding to a biosensor bound 50 bp DNA duplex representing the CCAAT box II in the promoter region of the penicillin biosynthesis gene *aatA*.

sic-region helix-loop-helix transcription factor AnBH1 to homogeneity. The AnBH1 binding site overlaps with the AnCF-binding site in the promoter of the penicillin biosynthesis gene *aatA* and acts as a repressor of the *aatA* gene. Furthermore we were able to reconstitute the AnCF complex *in vitro* and for the first time to measure the DNA-binding kinetics and affinity of the AnCF complex and AnBH1 to the CCAAT box in the promoter region of the *aatA* gene by surface plasmon resonance. (Figure 5)

Functional Analysis of Bone Morphogenetic Protein (BMP) Signaling

BMPs are a large group of secreted homodimeric proteins involved in the regulation of numerous biological processes during early embryonic development as well as organogenesis and tissue homeostasis. Almost all BMPs bind to receptor serine/threonine kinases and activate intracellular signaling molecules like the Smads. In contrast, the role of BMP-3 is not fully understood until now.

We analyzed the role of BMP-3 in the modulation of BMP-2 signaling. BMP-3 differs from the others members of the family with regard to its structure and its biological function. A special feature of BMP-3 is the existence of 2 potential proteolytic cleavage sites near the C-terminus resulting in BMP-3 containing a heparin binding site and BMP-3ΔN73 which lacks that site. By surface plasmon resonance analysis we could show that both BMP-3 variants are able to interact with the extracellular domain of the BMP type I receptor IA (BMPR-IA ECD) with a moderate affinity. Co-incubation of the promyoblast cell line C2C12 with BMP-2 and BMP-3 led to a further increase of alkaline phosphatase (ALP) activity in comparison to BMP-2 alone. In contrast BMP-2 and excess BMP-3ΔN73 showed reduced ALP activity. This surprising result might be in part caused by the heparin binding site of BMP-3 which has a higher binding affinity than the heparin binding site of BMP-2. In consequence, BMP-3

displaced BMP-2 from the extracellular matrix and produced a locally elevated level of BMP-2. Addition of heparin reduced the liberation of BMP-2 in the presence of BMP-3, because BMP-3 is bound by soluble heparin. The inhibitory effect of BMP-3ΔN73 on BMP-2 action is reduced by heparin. Due to the interaction of heparin with BMP-2 more free BMP-2 is available and therefore capable to bind to the BMP receptor complexes. Thus, BMP-3 has an indirect effect on BMP-2 receptor binding but BMP-3ΔN73 might directly bind to BMPR-IA.

In conclusion, we could show that BMP-3 interferes with the interaction of BMP-2 with receptor complexes containing BMPR-IA. The differential effects of BMP-3 and BMP-3ΔN73 in concert with heparin open a new view on the biological role of BMP-3 as part of the BMP signaling network.

Amyloid Fibril Formation and Characterization of Novel Amyloid Fibril Ligands

Amyloid fibrils are fibrous polypeptide aggregates that can be formed *in vitro* and under pathologic conditions, such as in Creutzfeldt-Jacob disease and Type II diabetes. In the case of Alzheimer's disease, these fibrils are formed from Aβ peptide. In collaboration with Dr. M. Fändrich from the Leibniz Institute for Age Research (FLI) we have developed recombinant expression systems that enables us to obtain large quantities of highly soluble Aβ(1-40) and human acute phase serum amyloid A (SAA) peptide.

Firstly, these wild type and mutated peptides were used for several *in vitro* studies in order to explore the kinetics and thermodynamics of aggregation and amyloid fibril formation. Our data show that the aggregation kinetics of Aβ(1-40) is controlled by stochastic nucleation events depending on peptide concentration. Secondly, we could establish a novel screening system which reproduces main features of *in vivo* amyloid formation using primary human macrophages and recombinantly generated Aβ(1-40) and SAA. This cell system allows to identify and validate effectors of amyloid formation that have an impact on the aggregation process or on the amyloid forming cells and can help to evaluate the mechanism of action, identify novel targets and discover the potential of clinically relevant compounds. Thirdly, we explored whether *in vitro* formed fibrils correspond structurally to those seen in the cerebral amyloid plaques of Alzheimer patients. To that end a novel VHH-domain was selected from a recombinant library of variable-heavy-domain (VHH) antibody domains and genetically fused with alkaline phosphatase (Dr. U. Horn, Department Pilot Plant for Natural Products). We find that this VHH-domain acts as a conformational antibody, i.e., it interacts strongly with mature fibrils, while it has no affinity for the monomeric peptide. (Figure 6) We conclude that the *in vitro* formed fibrils possess the same conformational characteristics as amyloid fibrils from cerebral Alzheimer plaques.

Bone Replacement Materials for Treatment of Osteoporosis

Bone Morphogenetic Proteins (BMP) induce and propagate skeletal wound healing. They enable osteoinductive mechanisms. Applied in combination with osteoconductive solid implant materials, BMP might accelerate and enhance bone regeneration even in individuals with compromised bone metabolism, such as osteoporotic or elderly patients. The choice of delivery system has a profound effect on the clinical outcome. Presently, soluble BMP released from rapidly degrading organic carriers such as collagen is favoured. The purpose of this project is the development of qualitative new bone replacement materials for local treatment of osteoporotic defects in humans by combining absorbable and non-absorbable inorganic carriers with bacterially produced rhBMPs. In collaboration with the DePuy Biotech GmbH Jena and the Research Department of the Orthopedic Surgery of the Friedrich-Schiller-University Jena we focused on the optimization of the BMP release kinetics from the carrier and on the minimization of the total BMP amount to avoid unwanted side effects from overdosing. The BMP release is influenced by at least three main parameters: degradative resorption of the carrier (as for collagen), solubility and affinity of BMP to a given carrier. We coated a hydroxyapatite carrier with our nonglycosylated rhBMP-2 and with a gly-

Figure 6
Atomic force microscopy image of biosensor bound Aβ(1-40) fibrils (A) and surface plasmon resonance sensorgrams of the association and dissociation of a camelid VHH-antibody to biosensor immobilized Aβ(1-40) fibrils or peptide.

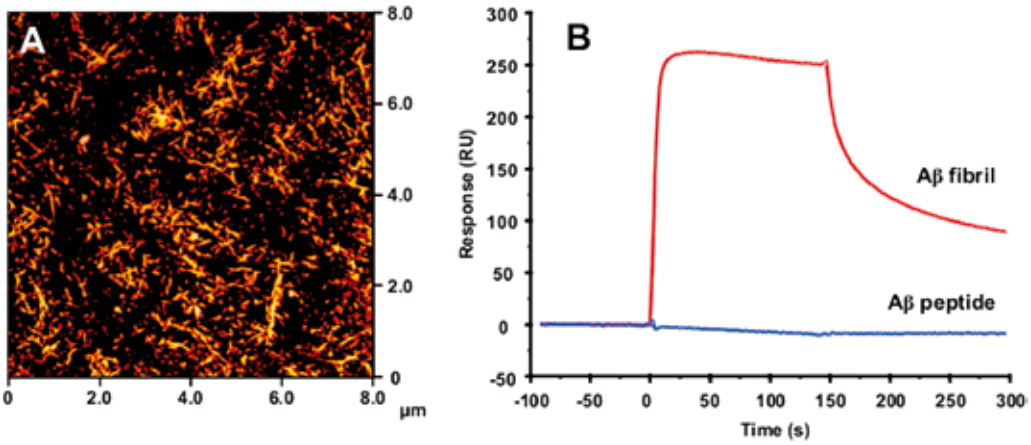
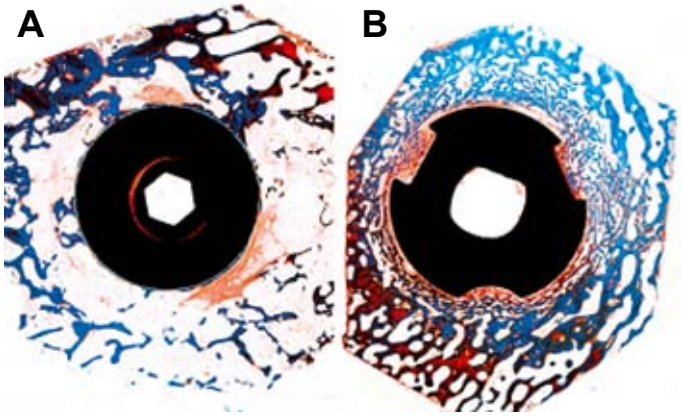


Figure 7
Bone ingrowth and gap healing of BMP-treated and non-treated test implants in an osteoporotic animal model. Histologic examination of an internal control site (A) and a rhBMP-2 treated site (B) from an animal 9 weeks after implantation.



cosylated commercial preparation. The release was measured with a novel sensitive ELISA based on a BMP-2 specific camelid VHH-antibody domain, which was selected by phage display from a fully synthetic library (Dr. U. Horn, Department Pilot Plant for Natural Products). The glycosylated variant displayed a rapid release kinetic whereas the nonglycosylated variant remained almost completely on the implant material. This suggests that nonglycosylated BMP-2 acts as a topical agent whereas the glycosylated variant may also function as a paracrine hormone. (Figure 7)

6 Systems Biology and Bioinformatics

Group Leader: Reinhard Guthke

Bio-Data Mining and Reverse Engineering

Mathematical modeling and systems biological research at the HKI is dedicated to the bioprocess research on the bioreactor, cellular and molecular level. The Group 'Systems Biology and Bioinformatics' is part of the 'Jena Centre for Bioinformatics" (www.imb-jena.de/jcb) and since 2003 of the German competence network "Systems of Live - Systems Biology" (HepatoSys, www.systembiologie.de)' as well as of the DFG Priority Program 1160 'Colonization and infection by human-pathogenic fungi'. R. Guthke was responsible for the Task Force "Intelligent Technologies for Gene Expression based Individualized Medicine" of the European Network on Intelligent Technologies (www.eunite.org). Today, R. Guthke co-ordinates the EU-funded focus group "Nature-inspired Modeling, Optimization and Control" (www.nisis.de). The aim of the Group for Systems Biology and Bioinformatics at the HKI is the discovery of structures and dynamics of biological systems. Process data are analyzed to generate computational models, which can be used to control or optimize experiments, biotechnical product forma-

tion and medical processes in diagnostics and therapy. The data mining and modeling by top-down approaches includes the analysis of data from DNA microarray and proteome data. The current research focuses on reverse engineering, i.e., the reconstruction of dynamic network models from time-resolved transcriptome and proteome data as well as – in future – metabolome and toponome data.

Dynamic Network Reconstruction from Gene Expression Data

Recently, we developed a reverse engineering (i.e., statistical network inference) strategy to reconstruct dynamic gene interaction networks that is based on both microarray data and available biological knowledge (Guthke R et al., 2005). The main kinetics of the response after a perturbation is identified by fuzzy clustering of gene expression profiles. The number of clusters is optimized using various evaluation criteria. For each cluster a representative gene with a high fuzzy-membership is chosen in accordance with available physiological knowledge. Seeking systems of ordinary differential equations, whose simulated kinetics could fit the gene expression profiles of the cluster-representative genes, identifies hypothetical network structures. Resampling methods are applied to validate the obtained dynamic network models. This novel reverse engineering algorithm was successfully applied to different transcriptome time series data sets, such as characterizing the response of

- *Escherichia coli* after induction of recombinant protein synthesis,
- human blood cells after infection by pathogenic *E. coli* (Guthke R et al., 2005),
- hepatocytes to LiCl to study the zonation of liver,
- hepatocytes to nutrient shift,
- patients to post-operative stress to evaluate sepsis,
- *Aspergillus fumigatus* towards temperature shift (Guthke R et al., 2006, *Lect Notes Bioinform*).

The last application is illustrated by the following: During the infection process the human-pathogenic fungus *A. fumigatus* has to cope with dramatic changes of the environmental conditions, such as temperature shifts. Recently, the complete genome sequence and gene expression data monitoring the stress response of *A. fumigatus* to a temperature shift from 30 °C to 48 °C were published (Nierman WC et al., 2005). We clustered the time series data, i.e., expression profiles of 1926 differentially expressed genes, by fuzzy c-means. The number of clusters was optimized using a set of optimization criteria (U. Möller/HKI Junior Research Group of Bioinformatics). From each cluster a representative gene was selected by text mining in the gene description. The expression profiles of these genes were simulated by a differential equation system, whose structure (Figure 8) and parameters were optimized minimizing both the number of non-vanishing parameters and the mean square error of model fit to the microarray time-series data. (Figure 9)

Integrative Transcriptome and Proteome Data Analysis

Transcriptome and proteome profiles in response to external stimuli and in different pathological states were analyzed by different methods for clustering, rule generation, network reconstruction and dynamic modeling. Automatic database searches were used to assist the interpretation and verification of the results. This was done in particular with respect to bacterial infections (*Streptococcus pyogenes*, (Klenk M et al., 2005)), cancer (leukemia, head & neck, colon, breast), rheumatoid arthritis, psoriasis and arteriosclerosis.

Within his PhD thesis, V. Monossov developed a novel rule-based method of gene expression data processing consisting of procedures for

- discretization and selection of variables (gene expression signals)
- rule-based knowledge extraction and decision making
- validation and analysis of rule-based knowledge using available knowledge (UniProt)

The novel procedure was applied to cancer classification based on three published gene expression microarray data sets: Leukemia data set (Golub et al., 1999), colon cancer set (Alon et al., 1999) and brain cancer data set (Pomeroy et al., 2002). Comparing the results

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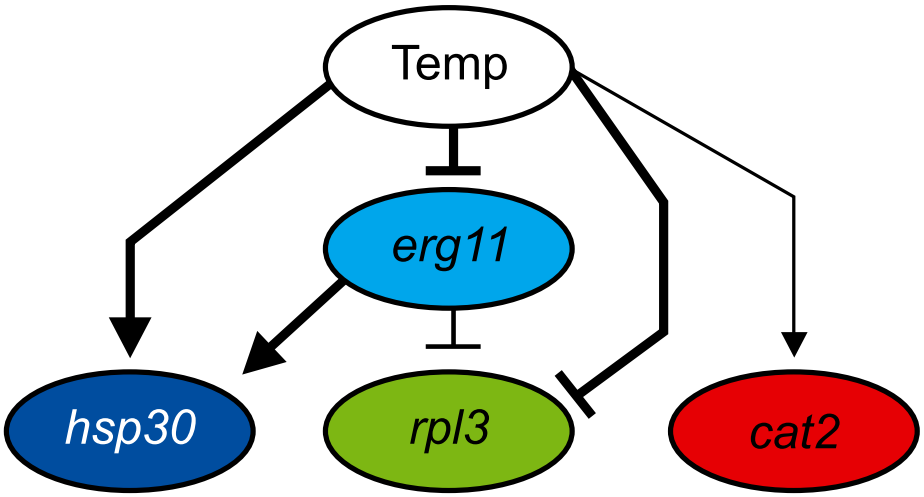

Figure 8
Structure of the dynamic model describing the temperature shift response of *Aspergillus fumigatus* identified by the model fit shown in Figure 9 (*Temp*: Temperature shift; *erg11*: cytochrome P450 sterol 14-alpha-demethylase, enzyme involved in ergosterol biosynthesis; *cat2*: catalase/peroxidase; *rpl3:* large subunit ribosomal protein L3; *hsp30*: heat shock protein class I). The arrows represent stimuli or activations. The T-shaped lines (⊥) represent inhibitions. The thick lines indicate the connections confirmed by resampling.

obtained with published classification results showed the superiority of the novel method.

First steps were taken in order to analyze also the proteome. In collaboration with F. v. Eggeling (FSU Jena) we had shown, that proteomic patterns during the development to cancer converges to common patterns (Mueller et al., 2006).

In a collaborative study with R. Kinne (FSU Jena) gene expression data from inflamed rheumatoid synovial tissue and purified synovial cell fractions of adherent macrophages, adherent fibroblasts and non-adherent cells from rheumatic patients and control persons were compared with respect to the expression patterns of relevant signaling proteins. This work is linked to the Collaborative Research Centre 604 (http://www.sfb604.uni-jena.de) studying signalling proteins such as phosphatases, PI3-kinases, histone deacetylases, NF-κB transcription factors as well as proteins involved in cell cycle control. The current research project within the Jena Centre for Bioinformatics is focused on application of this method of reverse engineering on transcriptome and proteome data measured in cells (fibroblasts) from patients suffering from rheumatoid arthritis (RA) and osteoarthritis (OA) after stimulating the cells by TNF-alpha and TGF-beta.

Gene expression profiles from peripheral blood mononuclear cells of 18 patients suffering from rheumatoid arthritis (RA) were monitored by partners in Magdeburg and Rostock using Affymetrix' Technology before as well as 3 and 6 days after anti-rheumatic drug administration using the TNF-alpha receptor fusion protein (Etanercept, Enbrel®). The responsiveness was quantified by a fuzzy approach using clinical parameters. We used supervised learning methods, e.g., Support Vector Machines (SVM), to identify gene sets that are differentially expressed in RA patients who have different responsiveness during therapy with the aim to individualize anti-rheumatic therapy (Glocker et al., 2006).

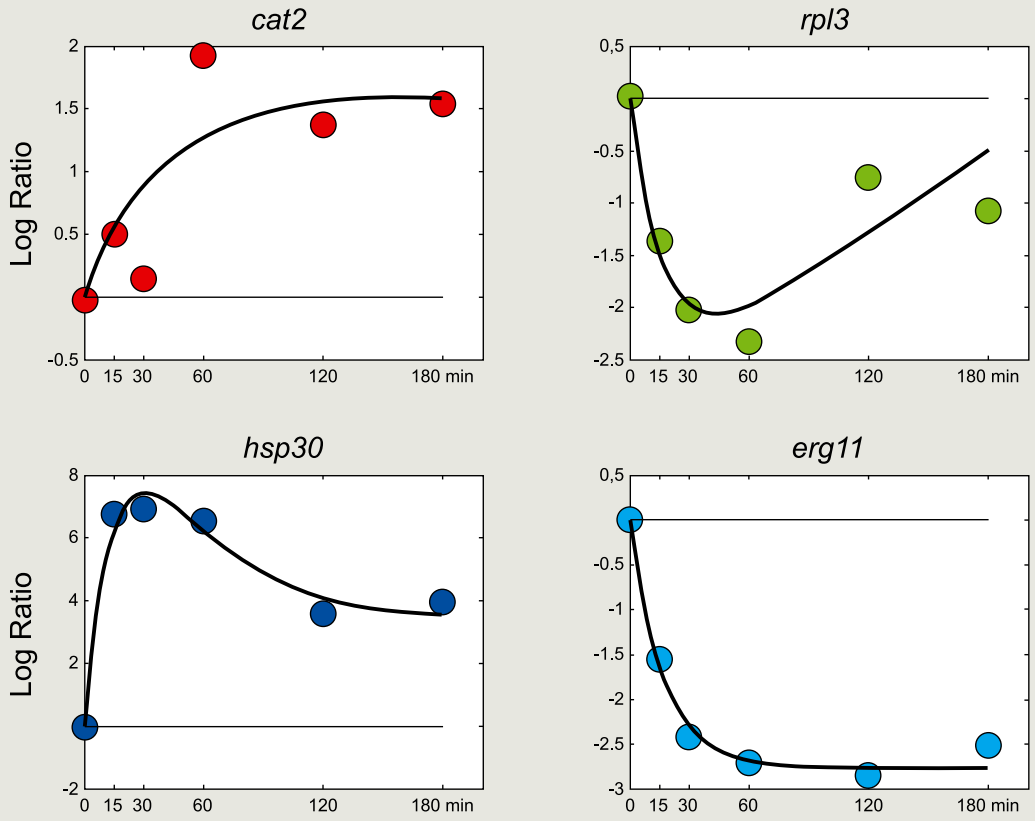

Figure 9
Measured (O) expression kinetics (log-ratios, from Niermann WC et al. 2005) for the genes selected as representatives of the 4 clusters and kinetics (thick lines) simulated by the differential equation system, whose network structure is shown in Figure 8.

Reconstruction of Metabolic and Gene Regulatory Networks in Liver Cells

In collaboration with the Charité Berlin (Campus Virchow) we analyzed the kinetics of 18 amino acids, ammonia and urea in 18 human liver cell bioreactor runs and simulated them by a two-compartment model consisting of a system of 42 differential equations (Guthke et al., 2006). The model parameters, most of them representing enzymatic activities, were identified and their values discussed with respect to the different liver cell bioreactor performance levels. The nitrogen balance based model was used as a tool to quantify the variability of runs and to describe different kinetic patterns of the amino acid metabolism, in particular with respect to glutamate and aspartate. This dynamic model is based on results of correlation and Bayesian network analysis performed to describe and predict the bioreactor performance and amino acid kinetics as well as its variability.

To investigate whether and how the Wnt/ß-catenin signalling pathway is involved in the zonation of liver, a culture of hepatocytes was stimulated by LiCl. Based on the gene expression profiles a hypothetic signal transduction network was constructed by a reverse engineering algorithm (Guthke et al., 2005; Zellmer et al., 2005).

7 Taxonomy

Group Leader: Ingrid Groth

Novel Taxa of Actinomycetes from Hypogean Environments

Abstract

Unexplored and unusual biotopes are still a rich source of novel taxa. Within the frame of a project funded by the EC we analysed the occurrence of heterotrophic bacteria in cyanobacteria dominated biofilms in Roman catacombs and in a Spanish cave. A preliminary phenotypic char-

Figure 10
Novel actinomycetes from hypogean environments

A *Catenulispora* sp., an acidophilic actinomycete representing a novel family and a novel suborder within the class *Actinobacteria*.

B *Myceligenerans crystallogenes* HKI 0369^T

C *Isoptericola hypogeus* HKI 0342^T

acterization of actinomycete isolates indicated that some of the strains could represent novel species. In collaboration with our EU-partner, the group of C. Saiz-Jimenez, Sevilla, Spain and with E. Stackebrandt from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ), Braunschweig, comprehensive polyphasic taxonomic studies were performed as a prerequisite for the description of these strains as novel species. As the result of our common studies six novel species of the genus *Agromyces*, one novel species of the genus *Isoptericola* and one of the genus *Myceligenerans* were published in the *International Journal of Systematic and Evolutionary Microbiology*.

In the search for novel producers of pharmaceutical important secondary metabolites the occurrence of actinomycetes on the acidic and heavy metal containing rocks of the former alum slate mine, Feengrotten (Saalfeld), was studied within the framework of the Basic Research Network Project of the HKI. The common application of phenotypic and molecular taxonomic methods allowed a rapid identification of the isolates and revealed the existence of interesting novel taxa in this artificial hostile biotope.

Novel Actinomycetes from Roman Catacombs

As result of the successfully finished EU-project, "Cyanobacteria attack rocks: Control and preventive strategies to avoid damage caused by cyanobacteria and associated microorganisms in Roman hypogean monuments" (EVK4-CT-2000-00028, 2000 – 2003), about 700 actinomycete isolates were provided for further scientific studies. These strains were phenotypically assigned to 27 genera of the order *Actinomycetales*. 287 strains were included in the screening for novel natural products of the HKI (see report MNF). Some of the isolates could not be unambiguously affiliated to established taxa. Therefore, in collaboration with our partners detailed taxonomic studies were

performed to clarify the taxonomic positions of these isolates. Based on our isolates it was shown that members of the genus *Agromyces* were very common in the studied hypogean environments and that the species diversity within these biotopes is very high. As the result of a three months stay of the PhD student Valme Jurado from Sevilla, Spain, six novel species of the genus *Agromyces* were commonly published.

With the descriptions of two further isolates as novel species of the genera *Isoptericola* and *Myceligenerans* we demonstrated that very rare actinomycetes were able to colonize these unusual biotopes. At the time of writing the description of *Isoptericola* this genus was represented only by a single strain (Stackebrandt et al., 2004). This strain was isolated from the hindgut of the Australian termite *Mastotermes darwiniensis* (Frogatt). Similarily, the genus *Myceligenerans* was also described for a single strain that was isolated from an alkaline salt marsh soil in China (Cui et al., 2004). By adding a second species we could show that members of this genus may not only occur in alkaline biotopes. (Figure 10)

Characterisation of Acidophilic and Heavy Metal Resistant Actinomycetes

Within the framework of the Basic Research Network Project the occurrence of actinomycetes on the acidic and heavy metal containing rocks of the former alum slate mine, Feengrotten (Saalfeld), was studied. To get access to actinomycetes being adapted to this hostile environment, modified methods for their isolation, laboratory cultivation and rapid identification had to be developed especially due to the limited growth of some isolates. Besides the established methods of phenotypic characterization molecular taxonomic methods were applied for the identification of isolates of such genera being important in this study (e.g. *Amycolatopsis*, *Kribbella*, *Pseudonocardia*, *Saccharothrix* and *Streptacidiphilus*). Members of these genera are already known as producers of industrial important secondary metabolites. Oligonucleotide primer pairs were designed which allow the strict taxon-specific amplification of 16S rDNA fragments. The genus specifity of the primers was evaluated in PCR assays using template DNA isolated both from marker strains and isolates from the Feengrotten and Roman catacombs. The latter were identified as members of the relevant genera by phenotypic and 16S rDNA sequence data. The use of specific probes as selective amplification primers allows not only a rapid identification of large numbers of environmental isolates but equally serves as a valuable tool in culture independent analyses of selected habitats in the search for novel producer strains.

To elucidate the diversity at the species and strain level a universal-primed-PCR protocol was established using the SpeedCycler technology. UP-PCR was introduced by Bulat et al. in 2000 as a whole genome analysis producing fingerprints, very similar to RAPD. However, this method is considered to be of a higher reproducibility than RAPD, as longer and semi-arbitrary primers are used. By using several primers in different combinations and ratios and by optimization of the PCR conditions a protocol was established that reproducibly generates high quality fingerprints unique for the relevant species or strain. The protocol was found to be universally applicable in actinomycetes below the genus level.

Phylogenetic analyses based on 16S rRNA gene sequences of selected isolates revealed that six of the isolates are closely related to one of the recently described novel phylogenetic lineage within the class *Actinobacteria* (Joseph et al., 2003; Busti et al., 2006). Some other strains are assumed to represent novel taxa of established genera. (Figure 11) The calculated positions in the phylogenetic tree as well as UP-PCR fingerprints (Figure 12) confirmed the genotypic separateness of our isolates from recognized species of the genera *Amycolatopsis* and *Kribbella*. Therefore polyphasic taxonomic studies are now in progress to describe these strains as novel species of the genera *Amycolatopsis* and *Kribbella*, respectively.

First results obtained from the primary natural product screening of the Feengrotten isolates revealed that the majority of these strains are able to produce bioactive compounds. Within the frame of a running Basic

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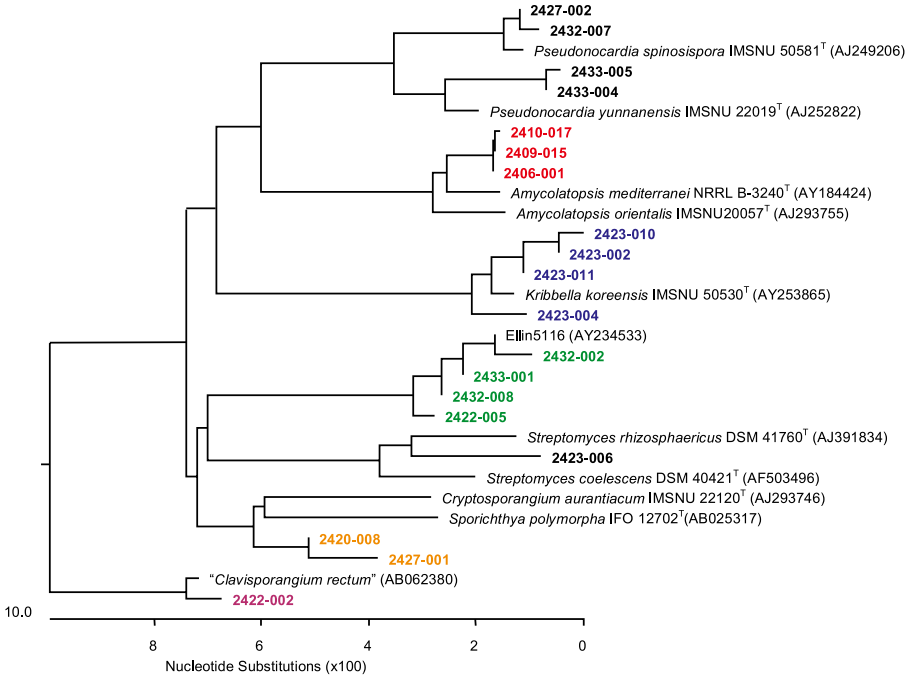

Figure 11
Phylogenetic tree based on 16S rRNA gene sequences showing the positions of our isolates from the Feengrotten, Saalfeld.

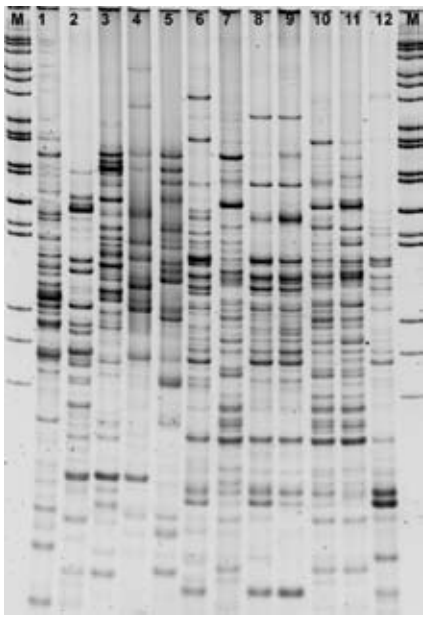

Figure 12
UP-PCR fingerprints of *Kribbella* strains
M: extended 100 bp Ladder (Roth),
1-3: *Kribbella* marker strains,
4-12: *Kribbella* isolates. 8% Acrylamide, Sybr Green staining

Research Network Project (Isabel Sattler) the chemical structures of these compounds will be elucidated.

Further activities of our research group were related to the occurrence of actinomycetes in indoor environments. Under the leadership of the Landesgesundheitsamt Baden-Württemberg the research group „Actinomyceten im Innenraum“ was founded in January 2004 to clarify the question, whether actinomycetes similar to fungi may represent a human health risk in contaminated indoor environments. The research group is composed of scientists from the universities Giessen and Göttingen, the DSMZ, the Umweltbundesamt (Berlin) and institutions of environmental medicine and diagnostics in Berlin, Düsseldorf, Karlsruhe and Aachen. Within the frame of this initiative we analysed indoor samples, organized a workshop in July 2004 in Jena and gave lectures about the biology and classification of actinomycetes. The common activities of this group resulted in a scientific project funded by the Umweltbundesamt. This project started in Dezember 2005.

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"Identification of virulence determinants of the human-pathogenic fungus *Aspergillus fumigatus* by proteome analysis"; DFG-Schwerpunkt „Kolonisation und Infektion durch human-pathogene Pilze" (BR 1130/9-1)
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„Evolution und Funktion vom cis-/trans-Elementen vom pilzlichen Sekundärmetabolismus am Beispiel der Penicillinbiosynthese"; DFG-Schwerpunkt „Evolution metabolischer Diversität" (BR 1130/8-1)
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Deutsche Forschungsgemeinschaft
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Selected publications
(HKI authors in bold)

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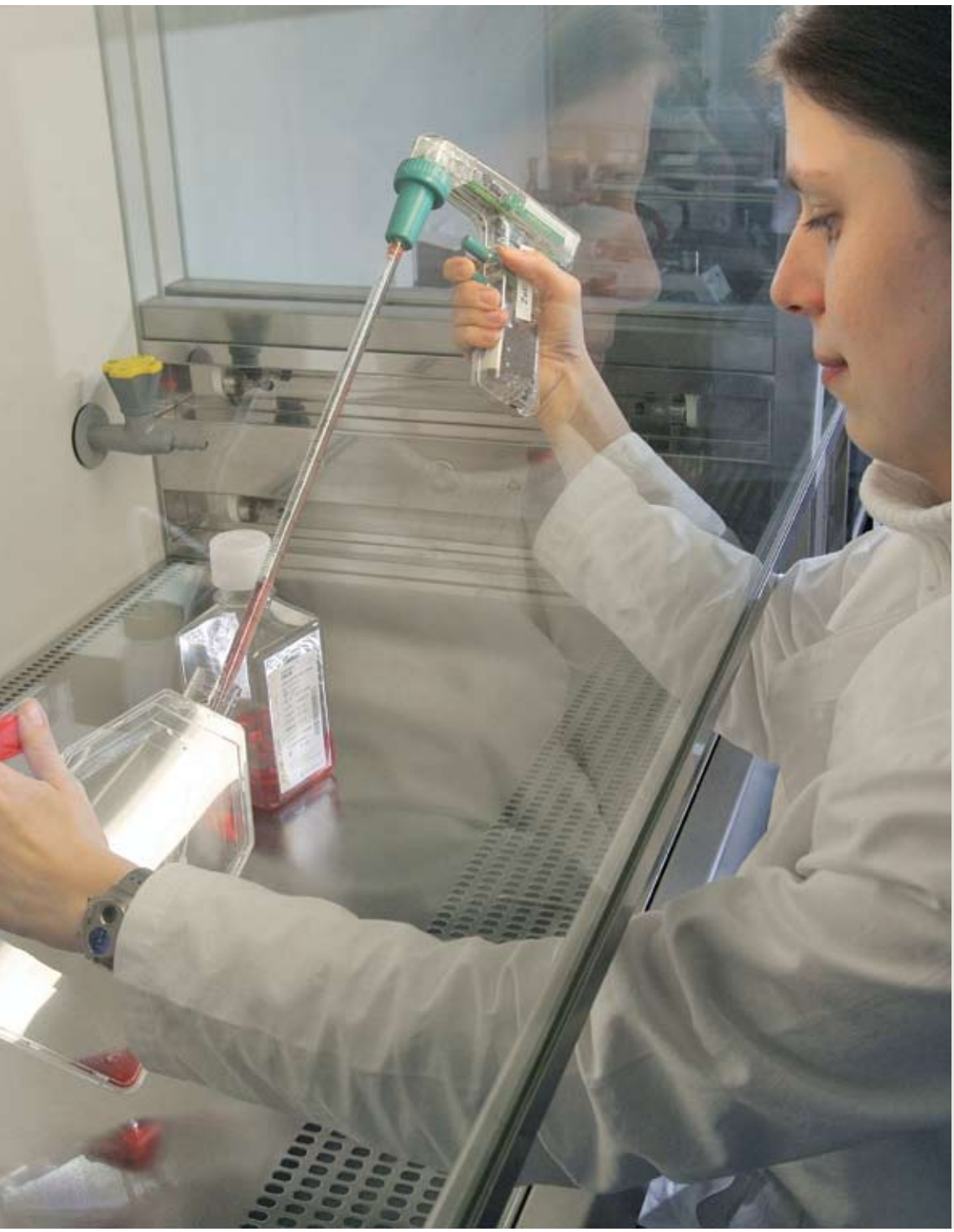


Department of Infection Biology

Department of Infection Biology

The department of Infection Biology focuses on the role of the complement system for immune evasion of pathogenic microbes, in particular pathogenic fungi and on immune dysfunction in form of autoimmune diseases. Pathogenic microbes evade host immune attack by expressing surface proteins that bind soluble host innate immune regulators. The identification and characterization of such microbial surface proteins reveals a general immune evasion mechanism and identifies novel virulence factors. The same soluble host immune regulators which are utilized by pathogenic fungi play a central protective action in the human host and maintain tissue integrity. Mutations of such regulators results in immundysfunction and autoimmune kidney diseases, such as membranoproliferative glomerulonephritis (MPGN), hemolytic uremic syndrome (HUS) and certain polymorphisms increase the risk for age related macular degeneration of the eye.

INTRODUCTION | EINLEITUNG

Head:
Prof. Dr. Peter Zipfel

Die Abteilung Infektionsbiologie beschäftigt sich mit der Rolle des Komplementsystems bei der Immunevasion von pathogenen Mikroorganismen, mit dem Schwerpunkt humanpathogener Pilzen 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 entscheidend sind. Die von pathogenen Mikroorganismen genutzten Komplementregulatoren des Wirtes spielen auch eine wichtige Rolle bei der Aufrechterhalung der Gewebsintegrität des Menschen. Mutationen in diesen Regulatorproteinen führen zur Immundysfunktion und Autoimmunerkrankungen der Niere, wie Membranoproliferativer Glomerulonephritis, der atypischen Form des Hämolytisch Urämischen Syndroms und bestimmte Polymorphismen des Gens erhöhen das Risiko für die altersabhängige Makuladegeneration des Auges.

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*

Abstract

The complement system provides a central and immediately acting defense system of the vertebrate host. Microbes, 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. Currently the first fungal CRASP-protein (complement regulator acquiring surface protein) has been cloned and recombinantly expressed. Antisera raised against the recombinant protein demonstrate expression on the yeast surface. The recombinant CRASP-1 protein binds several host plasma proteins, i.e. the immune 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. Structural and further functional characterization is aimed to identify how this yeast surface protein controls virulence and if these surface proteins serve as 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. (Figure 1) *C. albicans* and also *Aspergillus fumigatus* bind the human alternative pathway regulators Factor H and FHL-1 and the classical pathway regulator C4 binding protein (C4BP) to their surface. It is the interest of our group to identify and characterize these fungal surface proteins and to characterize the role of the surface attached host regulator for immune evasion.

In order to identify and clone *C. albicans* proteins that bind the human alternative pathway regulators Factor H and FHL-1, as well as C4BP three different approaches were used, i.e. (i) expression screening of a *candida lambda* gt11 DNA library, (ii) affinity chromatography in combination with mass spectrometry, and (iii) a proteomic approach using the expressed proteom of *Saccharomyces cerevisiae*. 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 protein 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; FHL-1 and plasminogen bind with similar intensities. When bound to candida CRASP-1 the host proteins maintain regulatory activities. Attached to CRASP-1 Factor H and FHL-1 regulate complement activation and inhibit 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

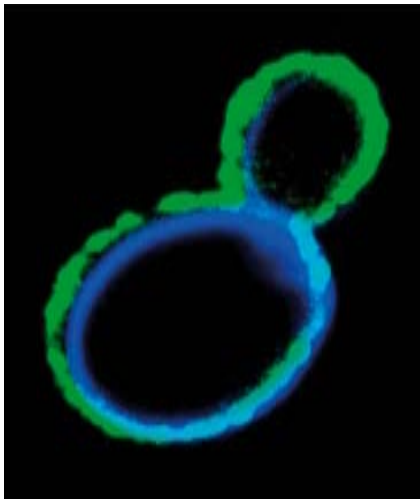


Figure 1
Immune- and complement evasion of *Candida albicans*.
The pathogenic yeast *Candida albicans* binds the human plasma protein complement Factor H (green) to its surface (blue) and utilizes the attached host protein for immune evasion.

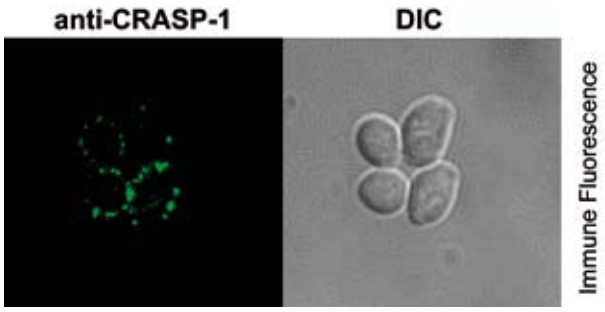


Figure 2
***Candida* CRASP-1 is surface exposed.**
Antiserum raised against the Candida CRASP-1 protein stains the surface of the yeast cell and is identified by green fluorescence.

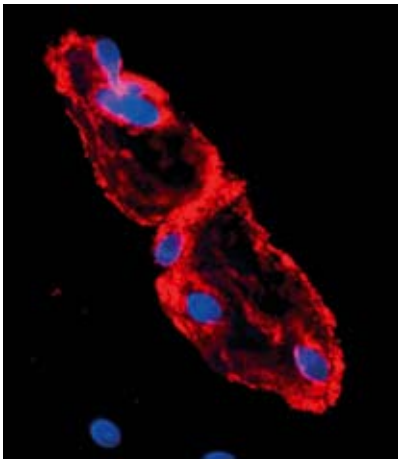


Figure 3
Phagocytosis of *Candida* cells by human epithelial cells.
Candia albicans (stained blue) is phagocytized by human epithelial cells, which are stained red. The hyphal form of the yeast is detected within the human cells.

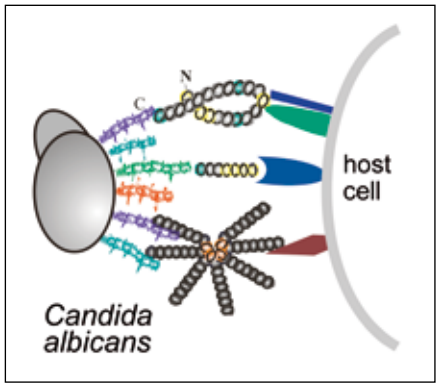


Figure 4
Multiple functions of attached host regulators.
The human pathogenic yeast *Candida albicans* expresses surface proteins which bind distinct host plasma proteins. The attached host proteins serve multiple functions. In addition to complement control they aid in the interaction and adhesion to host cells.

newly generated plasmin shows proteolytic activity and cleaves its chromogenic substrate S2251. Polyclonal antisera were raised against the purified CRASP-1 protein and were utilized to demonstrate surface expression of native CRASP-1 protein on yeast cells. (Figure 2)

In addition to complement control, the surface attached complement regulators Factor H and FHL-1 enhance adhesion to *Candida* cells and hyphae to host epithelial and endothelial cells. (Figure 3) When bound to the surface of candida both human plasma proteins enhance attachment to host cells in a role similar to fibronectin and fibrinogen. (Figure 4) Similarly the role of attached plasmin for interaction as well as degradation of the extracellular matrix is a relevant point to study.
In collaboration with the group of Professor A. A. Brakhage, Department of Molecular and Applied Microbiology, Jena, we investigated immune evasion of the human pathogenic yeast *Aspergillus fumigatus*. So far we have demonstrated that *A. fumigatus* similar to *C. albicans* binds four host immune regulators Factor H, FHL-1, FHR-1 and C4BP, as well as plasminogen to its surface.

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

Group Leader: Peter F. Zipfel

Immune Evasion Mechanisms of the Human Pathogenic Microbes

Abstract
The complement system provides a central and immediately acting defence system of the vertebrate host. 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. In addition to

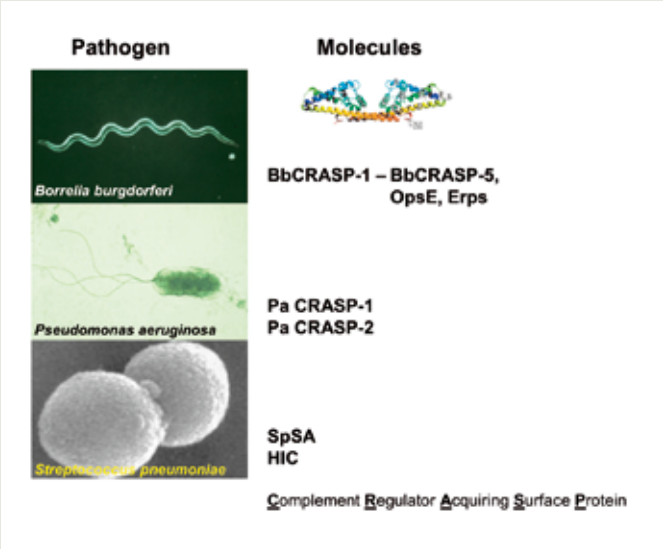


Figure 5
Immune and complement evasion of human pathogenic microbes.
Gram-negative bacteria as *Borrelia burgdorferi* and *Pseudomonas aeruginosa* as well as Gram-positive bacteria as *Streptococcus pneumoniae* express CRASP proteins (complement regulator acquiring surface proteins) that all bind the same human plasma proteins and that mediate immune evasion.

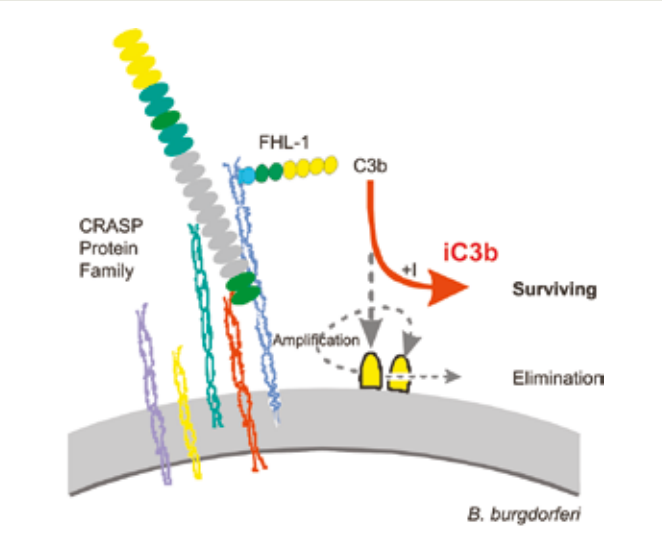


Figure 6
Microbial Immune evasion strategy.
A diverse group of microbes including Gram-negative and Gram-positive microbes express surface proteins which bind host immune regulators. The attached host immune regulators control complement activation directly at the microbial surface.

pathogenic fungi, 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 FHR-1 or the classical pathway regulator C4BP are bound. These microbial surface proteins have been identified and cloned from Gram-negative bacteria, including *Borrelia burgdorferi* and *Pseudomonas aeruginosa*, as well as Gram-positive *Streptococcus pneumoniae*. Binding of host immune regulators is a general, 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 CRASP-proteins from *Borrelia burgdorferi*, two CRASP- proteins from *Pseudomonas aeruginosa* and two CRASP from *S. pneumoniae*. (Figure 5) The characterisation of surface proteins, which are derived from a wide range of pathogenic microbes, allows the definition of a common immune evasion strategy 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. However there is no apparent sequence similarity and no common binding motive or pattern identified yet. It is a goal of our future work to characterize the common features of these diverse but functionally related microbial proteins.

A total of five CRASP-proteins have been identified in *Borrelia burgdorferi*. CRASP protein expression of *B. burgdorferi* is directly correlated with serum resistance and individual isolates express either a combination of all five CRASP-proteins or express different combination of these proteins. (Figure 6) A functional characterization shows that the various CRASP proteins show both unique, as well as common binding characteristics. CRASP-1

Figure 7
Structure of the Borrelial CRASP-1 protein.
CRASP-1 protein of *Borrelia burgdorferi* was crystallized and the structure of the protein was determined at X A. CRASP-1 shows a fold as a 'helical lollipop'. The dimeric protein at the surface of the bacterium has a cleft that can form a binding pocket for the host Factor H protein.

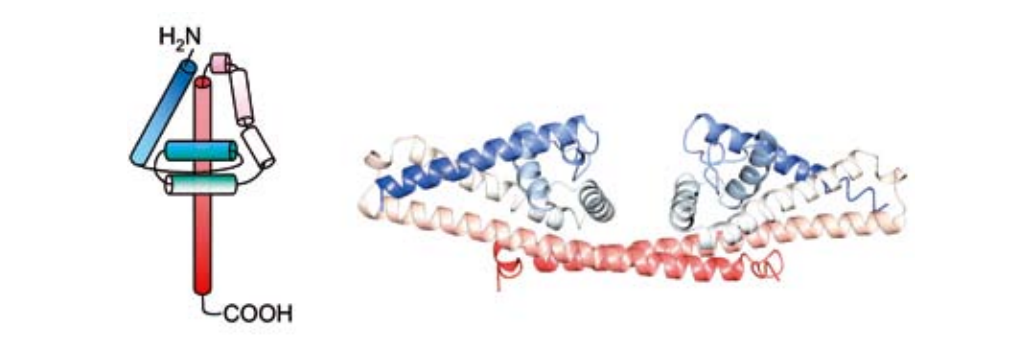
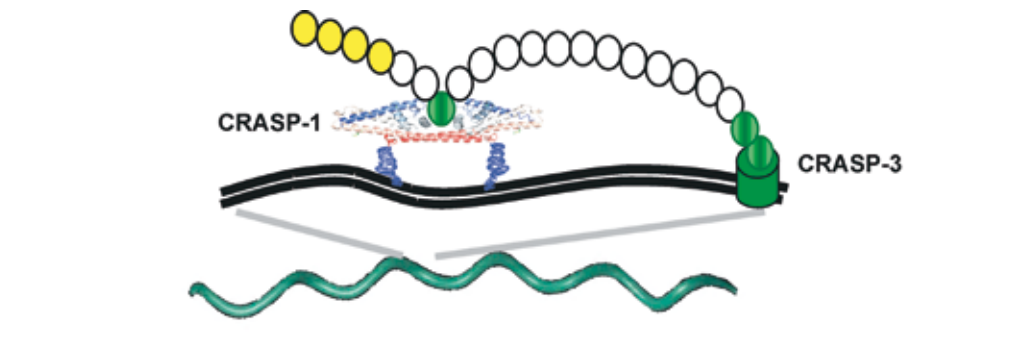


Figure 8
CRASP proteins mediate multiple contact points with host proteins.
Host regulators have multiple contact points to bind to the bacterial surface. By utilizing different binding sites and by expressing proteins that attach a host ligand at multiple sites the pathogen utilizes flexibility and can increase the affinity of the bound host regulator.



and CRASP-2 bind Factor H and FHL-1, but not FHR-1; in contrast CRASP-3, CRASP-4 and CRASP-5 bind Factor H and FHR-1, but not FHL-1. These binding specificities suggest a unique role for an individual CRASP protein in the immune response of *B. burgdorferi*.

The atomic structure of BbCRASP-1 protein has been determined. CRASP-1 as a monomer has a novel structure termed 'alpha helical lollipop'. In solution and on the surface of the pathogen CRASP-1 is a homo-dimer which forms a binding pocket for the host regulator. (Figure 7) The dimeric CRASP-1 protein is inserted into the outer membrane of the spirochete and attaches Factor H and FHL-1 *via* domain SCR 7. By expressing additional Factor H binding proteins at its surface the pathogen can attach Factor H at multiple points, which results in a firm attachment of the host regulator Factor H.

A detailed binding analyses shows that the host regulator Factor H has a two point for interaction and FHL-1 an one point interaction. Factor H binds to CRASP-1 and CRASP 2 with domain 7 and with the C-terminal recognition domain and in its bound conformation the N-terminal regulatory region located in the N-terminal four domains is still accessible, and functionally active. (Figure 8) By expressing multiple distinct surface proteins, borrelia provide several interaction sites for the host regulators, thus by increasing the number of attachment points the interaction and decoration with host proteins is increased, but at the same time a flexibility of various interaction sites is possible.

Particularly the serum resistant forms of the pathogenic bacterium *Pseudomonas aeruginosa* bind the host immune regulators Factor H and FHR-1. The immune evasion strategy of this Gram-negative bacterium is different, as it utilizes two separate strategies to control complement activation at its surface. The pathogenic forms use endogenous surface proteins that inhibit complement activation and inactivate C3b which is generated on the bacterial surface. In addition *P. aeruginosa* expressed surface

proteins that act as receptors for host immune regulators and that bind complement Factor H and FHR-1, an additional member of the Factor H protein family. After characterization of the immune evasion of this pathogen, two proteins were identified, which bind the two host immune regulators. The corresponding genes were cloned, the corresponding genes the proteins were expressed recombinantly and a detailed characterization of the two *P. aeruginosa* CRASP proteins is in progress.

Serum resistant Gram-positive *S. pneumoniae* express surface proteins that bind Factor H and FHL-1. The two CRASP proteins, 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 summary evasion of complement attack appears a common feature of pathogenic microbes. All analyzed pathogens utilize functionally related surface proteins, as they bind host plasma proteins and the complement regulators Factor H, FHL-1 and FHR-1. Several microbial CRASP proteins have been identified in pathogens. 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 CRASP proteins 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 CRASP proteins are highly polymorphic and show sequence variability between different strains,
(iv) attached host regulators are functionally active and attached to the microbe they inhibit C3b formation

The various CRASP proteins have the identical function as they bind the same host proteins. However so far a detailed sequence comparison of the individual proteins and of the localized interaction domains did no reveal a common sequence motive or conserved pattern of residues. As the diverse microbial proteins attach the host regulators at the same sites and domains it will be of great interest to identify the additional feature(s) which are common for this type of interaction.

3 Innate Immunity: Complement Dysfunction in Human Diseases

Group Leader: Peter F. Zipfel

Abstract

Defective regulation of the complement system results in immundysfunction and causes life threatening diseases, particularly kidney diseases. Genetic mutations of the human complement regulator Factor H are associated with severe kidney diseases in form of Membranoproliferative Glomerulonephritis (MPGN) and the atypical form of Hemolytic Uremic Syndrome (HUS). In general terms homozygous or compound heterozygous Factor H gene mutations result in MPGN, whereas heterozygous mutations result in HUS. The goal of this project is to understand and describe how single amino acid exchanges of the Factor H protein, affect protein function and cause disease. The detailed functional characterization of defective and mutated Factor H proteins derived from MPGN or HUS patients show either defective protein secretion which results in the absence or reduced plasma levels of the regulator, or in a secreted, functional defective mutant protein. Defective function are evidenced by reduced cell binding activities resulting in a reduced protective activity either at the surface of the glomerular basement membrane of the kidney in MPGN or on the surface of endothelial cells in HUS. These data already improved the diagnosis and provided novel ways for treatment and therapy of both forms of kidney diseases.

Membranoproliferative gomerulonephritis is a relative rare kidney disease which is defined by a thickening of the glomerular basement, due to deposits within the lamina dense of the glomerular basement membrane. (Figure 9) The disease is caused by the absence of Factor H in plasma or by Factor H mutations, that affect the function of the secreted protein. Defective Factor H secretion is explained on the genetic level

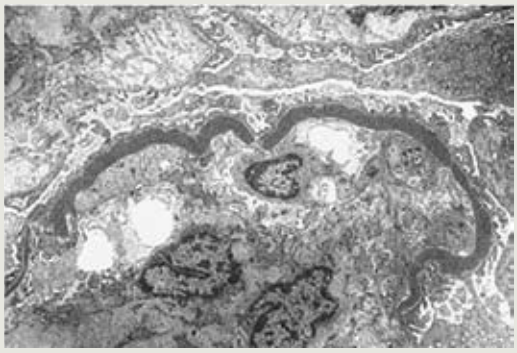


Figure 9
Renal biopsy of a patient with membranoproliferative glomerulonephritis (MPGN).
Typical findings of MPGN II (dense deposit disease) show thickening of the glomerular basement caused by deposits within the lamina dense of the glomerular basement membrane.

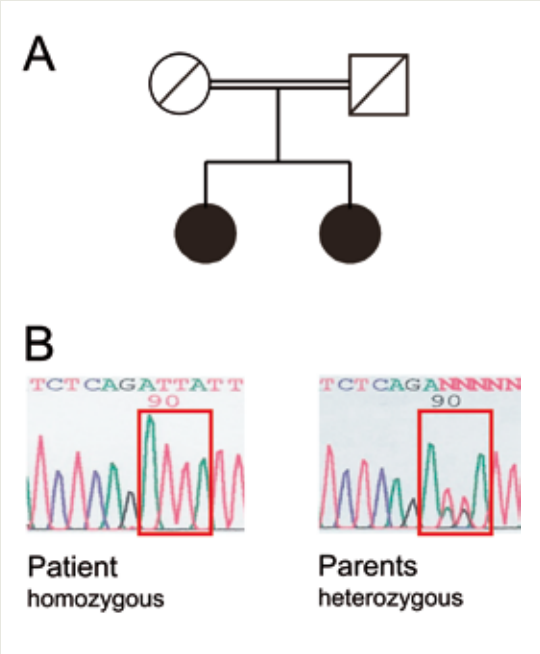


Figure 10
Pedigree of the family and Identification of a Factor H gene mutation in two patients with MPGN.
(A) Pedigree of the family, of which the two daughter developed MPGN. (B) Sequence analysis of Factor H identified a mutation in domain 4 of the protein (deletion of three base pairs: cDNA 743-745) resulting in a deletion of a Lys residue at position 224 (K_{224}). Two children of a consangouis parents are homozygous for the mutation and lack one single amino acid K_{224} Both parents are heterozygous for the mutation and have one defective and one intact allele.

by mutations which affect folding of the protein and thus result in a block of protein secretion. In addition to several human reports this scenario has been confirmed in animal models, either in Factor H deficient pigs, which represent natural mutants and provided the first animal model for MPGN and in Factor H knock out mice, which were genetically defined.

Recently we have identified a consangious family (Figure 10) which provide a second pathomechanism for Membranoproliferative Glomerulonephritis type II (MPGN II), which is caused by a mutant Factor H protein that is expressed in plasma. Genetic analyses of two patient's revealed deletion of a single Lys residue (K224) located within the complement regulatory region in domain 4 of Factor H. (Figure 11) This deletion resulted 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. The patients are daughters of consanguineous parents. As both patients but also their healthy mother were positive for C3NeF, the mutant Factor H protein is considered relevant for unrestricted activation of the disease causing activation of the alternative complement pathway. Replacement of functional Factor H *via* fresh frozen plasma was well tolerated, prevented so far disease progression in both patients and is in the long run 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 cohort and/or to identify additional genes which are associated with this form of kidney disease.

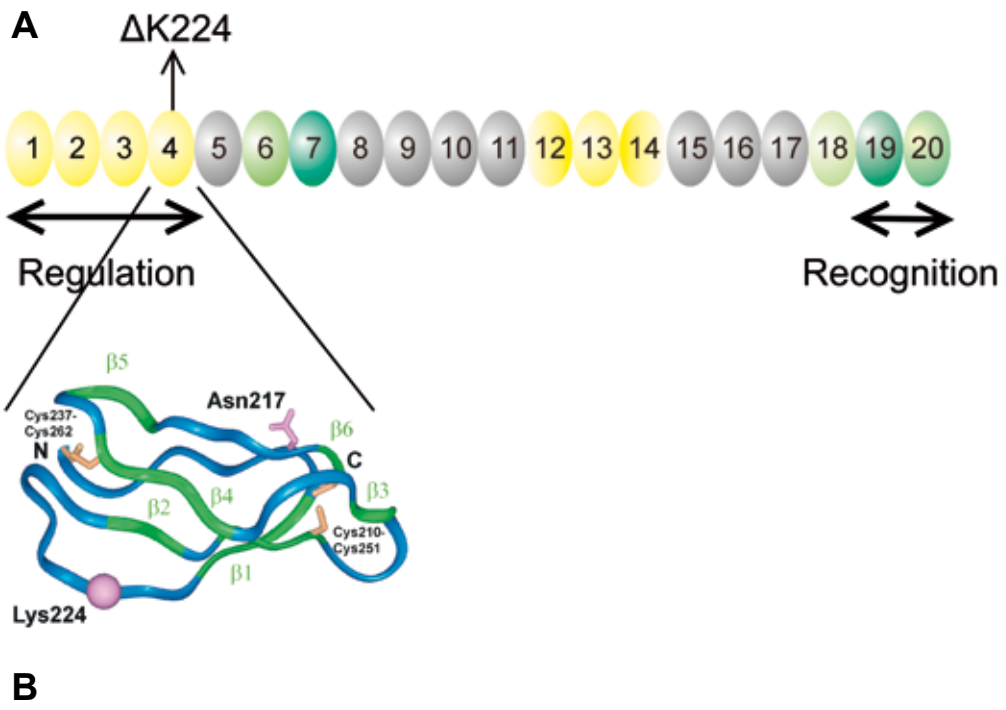


Figure 11
Position of the identified mutation within the Factor H protein.
A Factor H is shown with the 20 complement control protein modules, and the position of the deleted amino acid in module 4 is indicated. The position of the complement regulatory region in the N-terminus and that of the recognition region in the C-terminus are shown.

B Schematic structure of CCP 4 of Factor H. The Lys residue 224 is shown and highlighted with a purple circle. This part of the loop is positioned in the outside region of the CCP domain.

In atypical hemolytic uremic syndrome (aHUS) endothelial cell damage is associated with defective complement control. (Figure 12) Mutations in complement regulatory genes, including Factor H were reported in several aHUS patients and most of the Factor H mutations cluster in the C-terminal recognition domain of the protein (Figure 13) and result in reduced cell binding activity. (Figure 14) The role of Factor H mutations in aHUS is explained in cellular model system that allows to study the action of surface attached Factor H on endothelial cells. Upon complement activation on human umbilical vein endothelial cells surface attached Factor H show protective activity as evidenced by inactivation of surface deposited C3b, inhibition of MAC formation and reduced cell lysis.

This defect has been worked out for a patient who has a heterozygous mutation of the Factor H gene at R1203Stop, which introduces a premature stop codon in the most C-terminal domain, i.e. SCR 20. The mutant protein is expressed in plasma and identified as a truncated protein which shows increased mobility upon SDS-PAGE separation. The mutant Factor H protein was purified to homogeneity from the patients plasma and used for functional analyses in the endothelial cell system and showed severely reduced cell binding activity, which resulted in stronger complement activation at the surface of endothelial cells.

In addition complement activation at the cell surface was also enhanced in the presence of C-terminally binding mAbs which block Factor H binding to endothelial cells, but which leave the regulatory functions intact. Anti-Factor H autoantibodies are identified in plasma of several patients with HUS. A detailed mapping analysis with recombinant deletion fragments of Factor H showed that these autoantibodies bind within the C-terminal recognition domain of Factor H, i.e. to SCR 19 and SCR 20. Thus distinct scenarios, which lead to defective complement control result in HUS. Mutations in the Factor H gene can either block protein

Figure 12
Erythrocyte damage in patients with Hemolytic Uremic Syndome (HUS).
Damaged erythrocytes are detected in blood of patients with the atypical form of hemolytic uremic syndrome (HUS).

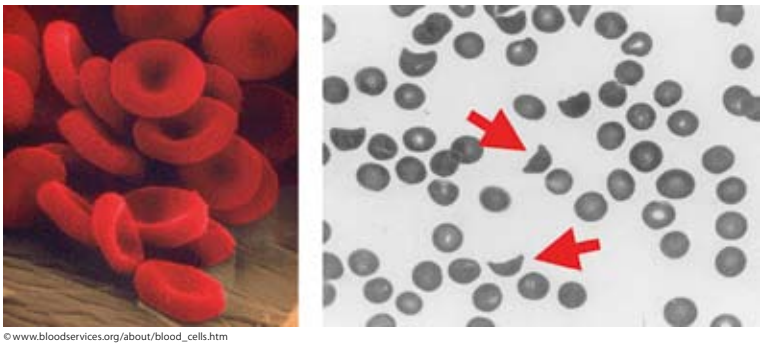


Figure 13
Factor H gene mutations are associated with Hemolytic Uremic Syndome.
Factor H gene mutations are identified in patients with the atypical form of hemolytic uremic syndome (HUS). The majority of mutations are heterozygous and most of the mutations are positioned in the C-terminal recognition domain of the protein in domains 19 and 20. About 25 % of patients with HUS show mutations in the Factor H gene.

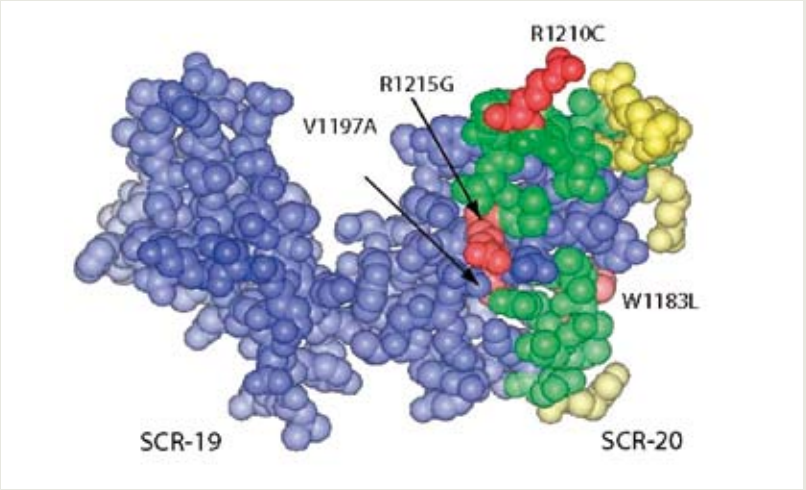
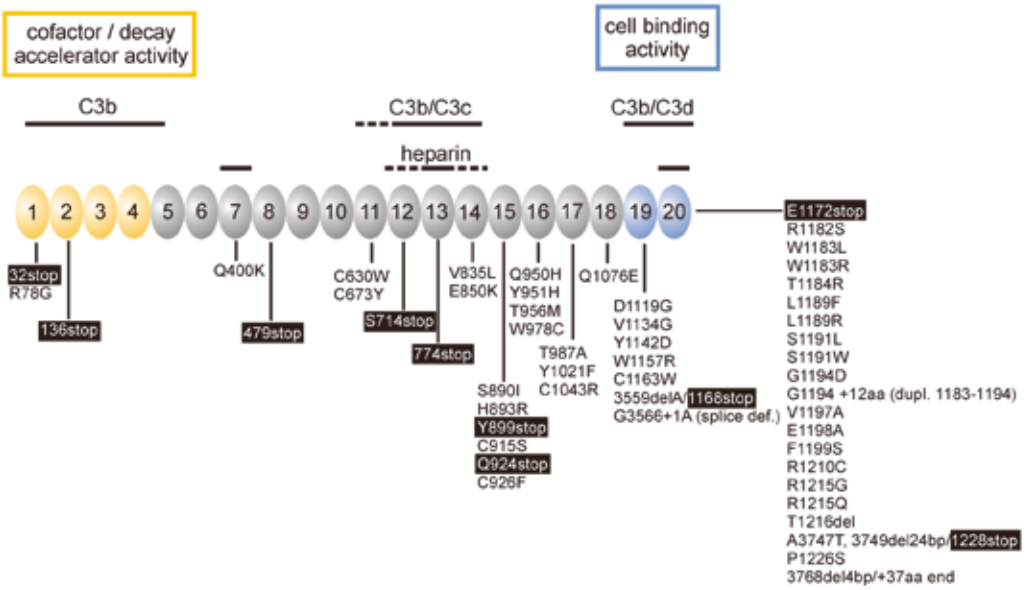


Figure 14
Position of mutated residues in the C-terminal domains of Factor H.
The structural model identifies the position and accessibility of the mutated residues. Two type of mutated residues are revealed. Type I residues are surface exposed and an amino acid exchange is likely to affect directly ligand interaction. Type II residues are buried and a mutation is expected to affect the overall architecture of the domain.



secretion and result in reduced plasma levels, or particularly that which cluster in the C-terminus affect the recognition function of the protein. In addition autoantibodies which bind predominantly within the C-terminal cell binding domain affect the recognition function of the protein. Thus complement regulation plays an important role to maintain integrity of cells and tissue surfaces. The soluble plasma protein Factor H binds to cells surfaces, and surface attached Factor H acts in concert with membrane-bound complement regulators on endothelial cells. This function explains how and why Factor H abnormalities, i.e., mutations of single amino acids, autoantibodies to Factor H, which cause defective cell binding activity, as well as reduced plasma levels, result in endothelial damage and allows progression to a HUS.

4 Adaptive Immunity

Group Leader: Christine Skerka

EGR Zinc Finger Transcription Factors as Regulators of Immune Response Genes

Abstract

Antigenic stimulation of T lymphocytes results in immune effector function, which is mediated by the release of cytokines and chemokines. Following antigen receptor signaling *de novo* cytokine and chemokine transcription is induced and regulated by nuclear transcription factors such as EGR, NF-κB, NF-AT, AP-1 and STAT. In general terms these DNA-binding proteins bind to their cognate promoters and initiate transcription of e.g. cytokine, chemo-kine and inflammatory genes. The precise mechanism how in lymphocytes individual DNA-binding transcription factors cooperate in order to control transcription of immune effector genes is currently unclear. Early growth response proteins (i.e. EGR 1 to EGR 4) are zinc finger DNA binding transcription factors that are transiently induced upon antigenic stimulation of T lymphocytes. We are interested to define how individual EGR transcription factors contribute to the signal dependent gene activation in lymphocytes. Individual EGR proteins bind to the promoter region of immune effector genes including IL-2, TNF-α and chemokine genes and mediate a strong transcriptional activity by physical and functional interaction with the immune regulators NFAT or NF-κB. The strong synergistic activities of EGR/NFAT and EGR/NF-κBp65 on gene transcription like TNF-α and ICAM-1 are completely abrogated by the EGR specific inhibitor protein NAB. NAB proteins bind to EGR as shown by pull down assays and by fluorescence resonance energy transfer (FRET). Thus Nab proteins repress the initial transcriptional activity induced by T cell stimulation and act as immune response modifiers by inhibiting the EGR/NFAT and EGR/NF-κBp65 activator complexes.

The four EGR- zinc finger transcription factors (EGR-1 to EGR-4) are transiently and coordinately induced in T lymphocytes upon antigenic stimulation and act as signal transducer from the cytoplasm into the nucleus. The individual EGR proteins regulate expression of immune effector genes like IL-2, TNF-α, the β chain of the IL-2 receptor, Fas and FAS-Ligand. In addition EGR proteins are expressed in distinct cell types and regulate transcription of a wide variety of genes, including genes involved in the control of cell growth and apoptosis. In T lymphocytes EGR proteins form stable physical complexes with other nuclear factors, like nuclear factor of activated T cells NFAT (Decker et al., 2003) and NF-κB proteins (Wieland et al. 2005) and the synergistic interaction of EGR and NFAT proteins enhances tissue specific expression of the immune effector genes IL-2 and TNF-α. Within the promoter regions of the two immune effector genes the specific EGR binding site is located directly upstream of the NFAT site. In T cells the EGR zinc finger proteins are simultaneously expressed and following signal transduction NFAT proteins are dephosphorylated and translocate into the nucleus. Thus particularly during the initial phase of T cell stimulation EGR proteins act in concert with NFAT and enhance transcriptional activation of immune effector genes.

Complex formation of EGR-proteins with both NF-κB proteins p50 and p65 was shown using recombinant and native proteins by protein

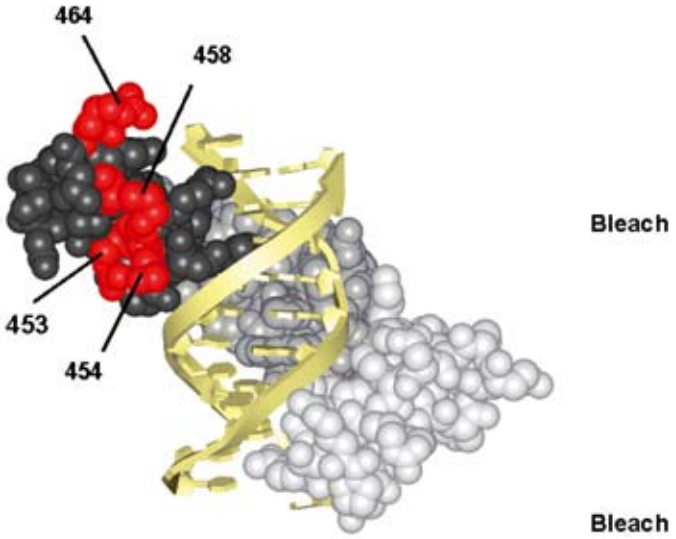


Figure 15
Location of the amino acid of the EGR-4 protein which interact with NF-κb p65.

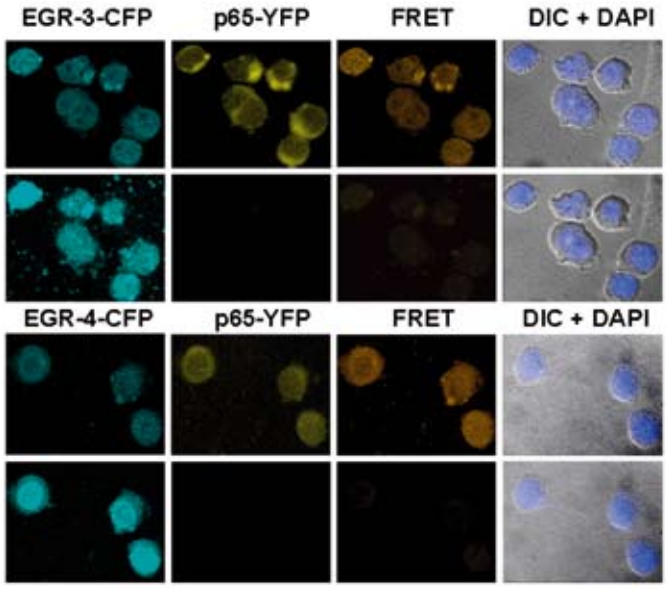


Figure 16
In vivo localization and interaction of EGR-3 or EGR-4 and NF-κB p65 proteins.

pull down assays, immunoprecipitation and by surface plasmon resonance (Wieland et al. 2005). The use of deletion mutants in transient transfection assays and peptide spot analysis identified the third zinc finger region of EGR-4 as the central domain for interaction and physical complex formation with NF-κB p65. The interaction domain is located within an alpha helical region and amino acids relevant for the interaction are surface exposed. (Figure 15)

The structure of the zinc finger region of the EGR-4 protein is derived from the crystal structure of the mouse EGR-2 protein (Zif268). Zinc finger I is shown in light grey, finger II in dark grey and finger III in black color. The amino acid residues of EGR-4 which are surface exposed and which interact with NF-κb p65 are shown in red.

The EGR/NF-κB complexes were identified within the nuclei of Jurkat helper T cells and a direct interaction of the nuclear EGR and NF-κB proteins was confirmed in vivo by fluorescence resonance energy transfer (FRET) assays (less than 10 nm). (Figure 16)

Jurkat helper T cells were transiently transfected with EGR-3-ECFP and p65-EYFP or EGR-4-ECFP and p65-EYFP expression vectors and fluorescence images were recorded after 24 hours. Images were acquired with filter settings specific for CFP, YFP or FRET signals (top panels) and after photobleaching of the acceptor protein (bottom panels). The two EGR proteins EGR-3-CFP and EGR-4-CFP are localized predominantly in the nucleus and p65-YFP is present in the cytoplasm. Upon photobleaching of the acceptor the fluorescence signal of the donor increases as crosstalk of the proteins is eliminated. The fluorescence signal of the acceptors, as well as the FRET signal is completely abrogated. Staining of DNA with DAPI indicates the nuclei of Jurkat T cells, that are shown by digital interference contrast (DIC) microscopy.

The transcription factor NF-κB plays a key

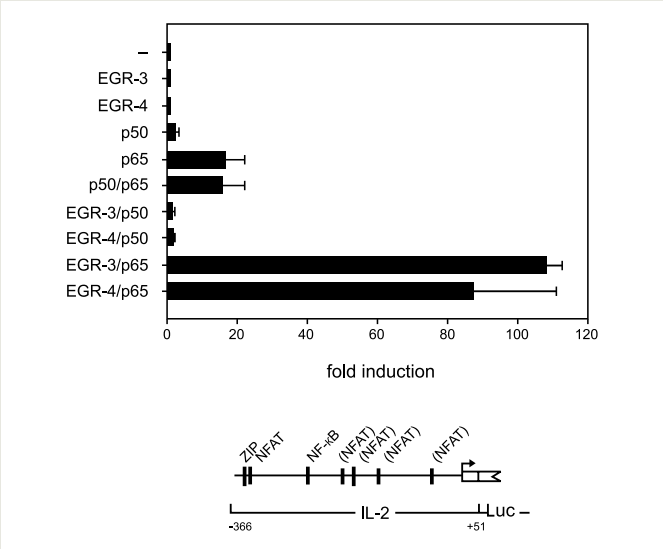


Figure 17
Transcriptional activity of EGR and NF-κB complexes on IL-2 expression. Human 293 kidney cells were cotransfected with an IL-2 reporter construct and various expression vectors. The transcriptional activity is shown as fold induction of the activity of the reporter construct alone, which was set 1. Mean values and standard deviations are shown from at least three independent experiments.

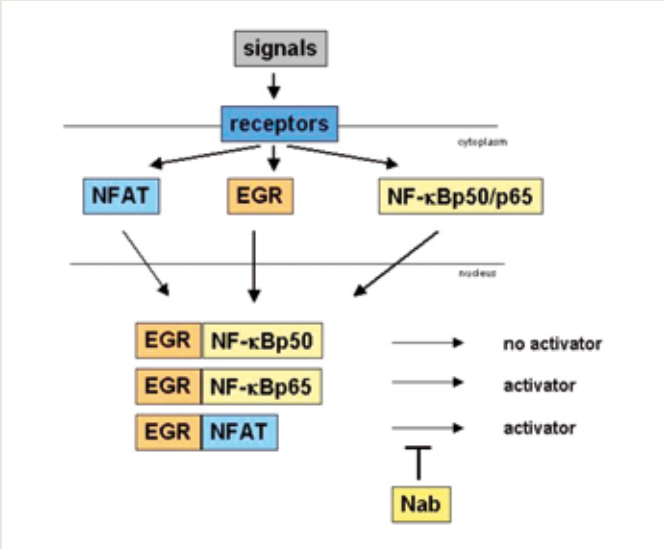


Figure 18
Signal transduction and regulation of proinflammatory gene transcription by EGR proteins.

role in inflammation and the immune response. The p50/p65 complex represents the most abundant dimeric form and the individual combinations of the subunits determine in immune cells specificity of transcription. Upon cell activation NF-κB translocates into the nucleus, binds to promoter sites and induces transcription of inflammatory mediators, such as cytokines (e.g. IL-1, IL-2, IL-6, IL-8), cell adhesion molecules (e.g. ICAM-1, VCAM-1, ECAM-1), chemokines (e.g. MCP-1, MIP1-α, MIP-1β) and immunoreceptors (e.g. IL-2R, Ig-κ light chain). The EGR/NF-κBp65 complexes strongly activated transcription of inflammatory gene promoters such as IL-2, TNF-α and ICAM-1 genes (Wieland et al. 2005) and this interaction is actually stronger than that of the NF-κB heterodimer p50/p65. (Figure 17) This synergistic interaction of EGR and NF-κb proteins explains how an ubiquitously expressed transcription factor like EGR regulates tissue specific gene expression in T-lymphocytes. EGR factors used alone are weak transcriptional activators, but in combination with NF-κBp65 become very potent transcriptional activators for cytokine genes like TNFα.

In T-lymphocytes and most likely in other immune effector cells, EGR zinc finger proteins act in concert with other specific and transiently induced transcription factors, such as NFAT and NF-κB proteins. This interaction controls the nuclear response of the signal transduction pathway from the T cell receptor *via* the cell membrane and cytoplasm into the nucleus and ensures specific and strong transcriptional activation of immune effector genes. The initial high transcriptional response of cytokine genes is terminated by specific inhibitor proteins which disrupt the EGR/p65 activator complex. NAB proteins, previously identified as specific inhibitor of EGR proteins, act as strong transcriptional repressor of EGR/p65 and EGR/NFAT mediated transcription. (Figure 18) Interaction was demonstrated for several EGR and NAB

proteins as well as NF-κBp65 and NAB proteins, indicating that NAB proteins inhibit transcriptional activity of the EGR/NF-κB complex by dissociating a specific nuclear activator complex.

Following antigenic stimulation of T lymphocytes signals are generated in the cytoplasmic domain of the antigen receptor and transcription factors are activated in the cytoplasma and translocate into the nucleus. Within the nucleus EGR proteins interact and form stable physical complexes that imitate and control transcription of immune effector genes. Transcription of immune effector genes is controlled and likely inhibited by Nab proteins, which most likely inhibit synergistic transcriptional activity by dissociating the EGR/NFAT or EGR/p65 complexes.

5 Bacteriology

Group Leader: Ute Möllmann

New Approaches for the Therapy of Infectious Diseases Caused by Critical and Resistant Pathogens

Abstract

The interest of the Bacteriology group focuses on new strategies for the therapy of infectious diseases. Different approaches are used, such as the identification and evaluation of new target structures, development of target based assay system to allow screening of low molecular weight effector compounds or the identification of novel lead compounds. By exploring alternative routes for drug delivery, existing antimicrobials will be 'reactivated' in order to overcome existing resistant mechanisms, like the permeability barrier of the bacterial envelope. These approaches are applied on pathogens like *Mycobacterium tuberculosis*, multiresistant staphylococci, vancomycin resistant enterococci, *Candida* and *Aspergillus* strains. The research of the bacterial group is integrated and actively linked with the Internal Product Line of the Institute.

Identification of Novel Drug Targets from Protein Linkage Maps

As the sequence of the entire *M. tuberculosis* genome is currently available a functional genomic- and a protein linkage approach is used to identify novel drug targets. Protein-protein interaction studies are performed in order to identify molecules relevant for intraproteome as well as host-pathogen interactions of *M. tuberculosis*.

Protein interaction is central for many cellular processes like signal transduction, transcription, replication, and also important for pathogenicity. Due to the limited knowledge of protein-protein interactions in *M. tuberculosis* and between the pathogen with its host, we are characterizing the protein-protein interactions network of the pathogens in order to open new avenues for diagnosis, prevention and treatment of diseases caused by *M. tuberculosis*.

Protein interaction of the *M. tuberculosis* proteome is studied by the yeast two-hybrid system. Selected open reading frames of *M. tuberculosis* are used as baits to screen prey libraries derived from *M. tuberculosis* or its mammalian host. With this approach novel interacting protein pairs of *M. tuberculosis* proteins are identified. Following a detailed characterization of the interaction in biochemical terms, interacting proteins relevant for pathogenicity and survival of the bacterium will be used to develop screening systems. Thus is expected to identify novel inhibitor molecules, that will serve as potential drug candidates against tuberculosis. With the same approach additional interacting proteins were identified for exported, as well as or surface exposed *M. tuberculosis* proteins and host proteins. An attractive pair of interacting proteins is the secreted *M. tuberculosis* ESAT-6 protein and the intracellular human syntenin-1. This interaction allows to study the role of the mycobacterial virulence factor ESAT-6 during the infection process. This interaction was confirmed by biochemical means using recombinant ESAT-6 and human syntenin-1, for *in vitro* protein interaction studies and surface plasmon resonance, and by co-purification analysis of the mycobacterial expressed ESAT-6 and macrophage

derived syntenin-1. The interaction domains were localized by two-hybrid studies using truncated derivatives of both proteins and by peptide spot analysis. Two domains of each protein mediate the ESAT-6/syntenin-1 interaction. The C-terminus of ESAT-6 binds to the PDZ-domains of syntenin-1 and the N-terminus of ESAT-6 binds to the N-terminus of syntenin-1. Thus, the host protein syntenin-1 represents a possible cellular receptor for the mycobacterial protein ESAT-6.

Internal Product Line (IPL)

The Bacteriology group coordinates the activities of the HKI Internal Product Line. This includes the organization of the HKI-strain collections, extract pools, natural product databases, product lines and screening procedures, as well as the coordination of secondary screening approaches and transfer of techno-logy or compounds transfer to external partners and companies.

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„Molekulare Genetik und Pathogenese des Hämolytisch-Urämischen Syndroms im Erwachsenenalter“ (ZI 432/7-1)
Peter F. Zipfel

Deutsche Forschungsgemeinschaft
„Molekulare Genetik und Pathogenese des Hämolytisch-Urämischen Syndroms im Erwachsenenalter“ (ZI 432/7-3 7-4)
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“Immune evasion mechanisms of the human pathogenic yeast *Candida albicans*”;
DFG-Schwerpunkt „Kolonisation und Infektion durch humanpathogene Pilze“ (ZI 432/9-1)
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Deutsche Forschungsgemeinschaft
„Funktion von Faktor H und Mitgliedern der Faktor-H-Proteinfamilie bei der Komplementregulation und Zellinteraktion“ (ZI 432/5-1)
Peter F. Zipfel

Deutsche Forschungsgemeinschaft
„Funktion von Faktor H und Mitgliedern der Faktor-H-Proteinfamilie bei der Komplementregulation und Zellinteraktion“ (ZI 432/5-2)
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„Multiple Funktionen der Phosphatidylinositol 3-Kinase VPS34P der humanpathogenen Hefe *Candida albicans*“, Sonderforschungsbereich SFB 604 „Multifunktionelle Signalproteine“ (SFB 604-02)
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Industry
Henkel KGaA
„Suche nach Inhibitoren der Adhäsion und Vps34p-Enzymaktivität von *Candida albicans*“
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“Role of Complement Regulator Factor H in Membranoproliferation”
Peter F. Zipfel

National Institutes of Health, USA
“Novel Derivatization / Functionalization of Natural Products” (1R01GM075855-01)
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Thüringer Ministerium für Wissenschaft, Forschung und Kunst
„Innovative Target-orientierte Testsysteme zur Suche nach Wirkstoffen zur Therapie von Mykosen des Menschen“ (B 307-01009)
Peter F. Zipfel

Foundation for Children with atypical HUS
“Role of Factor H gene mutations for the pathophysiology of HUS”
Peter F. Zipfel

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(HKI authors in bold)

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Collaborations

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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 modern aspects of basic and applied research. During the last two years several diploma and doctoral

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 Industrien. Die Erfahrungen und Kenntnisse, die wir aus unseren Arbeiten gewinnen, erlauben es uns, Studenten theoretisch und praktisch mit modernsten Aspekten der Grundlagenforschung und angewandten Wissenschaft zu konfrontieren. So hatten wir in den letzten zwei Jahren wiederum mehrere Diplomanden

students have graduated successfully at our department. In this context we congratulate Steffi Herold, who in great competition received the medac award for best thesis work on negative regulation of mammalian stress response including publication in the prestigious journal "Molecular Cell". Within our technological framework we have had some real success on patents related to rapid heat block thermocycling of small samples. The issuance of these internationally coveted patents by the European and U.S. Patent and Trademark Offices 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. A first commercial device, i.e. the SpeedCyler and corresponding consumables are produced and sold worldwide by Analytik Jena.

und Doktoranden in unserer Abteilung, die ihre Studien mit Erfolg abschliessen konnten. In diesem Zusammenhang möchten wir Frau Steffi Herold gratulieren, die trotz großer Konkurrenz mit dem medac-Forschungspreis für die beste Dissertation über negative Regulation von Stressantworten in Säugern und eine daraus resultierende Publikation in dem angesehenen Journal „Molecular Cell" ausgezeichnet wurde.

Guten Erfolg konnten wir im Hinblick auf Rapid RCR verzeichnen. Sowohl das Amerikanische als auch das Europäische Patentamt haben unsere international begehrten Erfindungen anerkannt. Somit verstärken unsere Patente das HKI-Patentportfolio zum Thema „Hochtechnologie".
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. Ein erstes Gerät, der so genannte SpeedCycler, mit dazugehörenden Verbrauchsmaterialien wird von Analytik Jena auf Grund unserer Patente hergestellt und weltweit vertrieben.

Scientific Projects

1 Apoptosis Inhibition by *Chlamydia pneumoniae* Infection

Group Leader: Hans Peter Saluz

Cellular Stress and Apoptosis

Within the framework of this project, we investigate the regulation of cell suicide in human cells upon *Chlamydia pneumoniae* infection. *C. pneumoniae* is one of the most widespread human pathogens. It is the third most common etiologic agent of respiratory tract infections like trachoma, sexually transmitted disease, bronchitis, pneumonia and others. It is suggested that more than 70% of people throughout the world have been exposed to that pathogen during their lifetime (Saikku et al., 1992). No *C. pneumoniae*-infected cells studied so far underwent apoptosis (Rajalingam et al., 2001). Therefore, we investigate anti-apoptotic mechanisms of *Chlamydia pneumoniae* to elucidate how this intracellular bacterium circumvents apoptosis in its host cells.

Apoptosis is a mechanism of cell suicide in multicellular organisms to remove unwanted cells. This process is mediated by proteolytic caspases triggering cell death by cleaving specific proteins in cytoplasm and nuclei. Certain viruses and intracellular bacteria are capable to circumvent host-cell suicide, *Chlamydia* is a wide spread pathogen causing many severe diseases, like trachoma, sexually transmitted disease, respiratory tract infections and others. It is an obligatory intracellular gram-negative bacterium that undergoes a biphasic growth cycle with distinct morphological and functional properties: the elementary body (EB) is the non-replicative infectious extracellular form and the reticulate body (RB) is the replicative non-infectious, intracellular form. One species, *Chlamydia pneumoniae*, is found in approximately 50% of adults. Due to its broad distribution, its responsibility for respiratory tract infections like bronchitis and pneumonia, and its association with atherosclerosis, it became of great medical and industrial interest.

For a successful infection C*hlamydia* binds to host-cell receptors, internalized, then enclosed within membrane-bound compartments, the "inclusions", and finally transported to a perinuclear location. *Chlamydia* enters host cells either by actin-dependent phagocytosis and/or clathrin-dependent endocytosis. The bacteria survive within the inclusions by inhibiting fusion with lysosomes. Furthermore they are capable to influence host cell suicide, i.e. no alls infected by *Chlamydia pneumoniae*-infected cells studied so far underwent apoptosis (Rajalingam et al., 2001). First, we have confirmed the above findings also for endothelial cell lines by DNA-fragmentation assays, 4',6-diamidino-2-phenylindole (DAPI) staining approaches and terminal deoxynucleotidyl transferase mediated dUTP nick-end labeling (TUNNEL) experiments. Furthermore, our investigations by differential gene expression studies using chip technology and functional protein analysis to elucidate how host-cells infected by *Chlamydia pneumoniae* escape suicide, revealed the initiation of the apoptosis death-receptor pathway by complex formation between dead receptor Fas and its ligand Fas-L thus initially activating initiator caspase cascade. However, no activation of extruder caspase 3 could be observed. Further analysis revealed a novel potential chlamydial caspase inhibitor. At present we subject this protein to MS analysis and relevant functional studies to elucidate the precise inhibitory mechanism.

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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 tumor marker due to its overexpression in various malignancies. On the other hand, over expression of genes encoding antiapoptotic proteins leads to the survival of leukaemic cells *via* exclusion of apoptosis. Therefore, we investigate the relationship between PRAME over expression 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.

The preferentially expressed antigen of melanoma *(PRAME)* was identified as a gene encoding a 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 M, 2001), 62% of 31 childhood AML patients, 42% of 21 childhood ALL patients (Steinbach D, 2003) and 26% of 58 patients with CLD (Proto-Siqueira R, 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 M, 2001).

We investigate the relationship between PRAME over expression 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.

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. 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

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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 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 funghi 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 approxiamtely 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 funghi 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 funghi 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 antitumor 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 funghi 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 the cell lines expressed antiproliferative effects.

Chemical analysis revealed polysaccharides of

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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 Topoisomerase IIβ Interacting Protein 1: A Link to DNA Repair, Replication and Apoptosis

Group Leader: Frank Hänel

DNA Repair, Replication and Apoptosis

Within the framework of this topic we study protein-interactions involved in stress response in human cells. Stress factors can be DNA damaging agents such as UV irradiation or humanpathogenic microorganisms. The aims of these studies are investigation of cellular mechanisms of stress response like DNA damage checkpoints, identification and validation of new targets for anti-cancer or anti-microbial therapy. New two-hybrid interactions were validated in human cells and used for the search of low-molecular weight substances which inhibit these interactions.

We showed that the Myc oncoprotein represses initiator-dependent transcription through the POZ-domain transcription factor Miz-1. Additional studies revealed that transactivation by Miz-1 is negatively regulated by association with the topoisomerase IIβ binding protein (TOPBP1); UVB irradiation downregulates expression of TOPBP1 and releases Miz-1. Miz-1 binds to the p21cip1 core promoter and is required for upregulation of p21cip1 upon UVB irradiation. Using both *c-myc* deficient cells and a point mutant of Myc that is deficient in Miz-1 dependent repression, we showed that Myc negatively regulates transcription of p21cip1 upon UVB irradiation and facilitates recovery from UV-induced cell cycle arrest through binding to Miz-1. We proposed that TopBP1 (as a sensor) and Miz-1 (as an effector) are involved in a DNA damage checkpoint (Herold et al., 2002). Therefore, we are investigating the function of TopBP1 within this hypothetical DNA damage checkpoint signaling pathway mainly by the search and characterization of further unknown TopBP1-interacting proteins (DFG: HA 2519/2-3/4). TopBP1 was initially found in a two hybrid screen with DNA topoisomerase IIβ as a bait. TopBP1 contains eight BRCA1 C-terminal (BRCT) motifs and interacts with several other proteins, including human papilloma virus type 16 (HPV16) transcription/replication factor E2 and others. It appears to be involved in DNA replication because incubation of an antibody against the sixth BRCT motif of TopBP1 inhibits DNA replication in an *in vitro* HeLa nuclei replication assay. TopBP1 shares sequence and structural similarities with the fission yeast Rad4/Cut5 protein, a checkpoint Rad protein involved in cellular responses to DNA damage and replication blocks. Upon γ-irradiation, TopBP1 colocalizes with Nijmegen breakage syndrome 1 (NBS1), BRCA1, and p53 binding protein 1 (53BP1) in the ionizing radiation-induced foci representing stalled replication forks. In addition to the control of DNA replication, TopBP1 is also required for cell survival. Inhibition of TopBP1 expression by antisense Morpholino oligomers or by siRNAs directed against TopBP1 induces apoptosis. Thus, TopBP1 is involved in several important aspects of growth control (Garcia et al., 2005).

Our *in silico* analysis of the TopBP1 protein sequence revealed that TopBP1 contains a Poly(ADP-ribose) (PAR) - binding motif. As the protein PARP-1 is also involved in processes such as DNA repair and replication, we investigated the interaction between TopBP1 and PARP-1 *in vitro* and *in vivo*. We demonstrated that PARP-1 interacts with the 6th BRCT domain of TopBP1 *in vitro*. Furthermore, PARP-1 coprecipitates with TopBP1 antibody *in vivo* in HeLaS3 cells; both proteins colocalize in the nucleus of the same cells. In addition, TopBP1 negatively regulates the auto-(ADP-

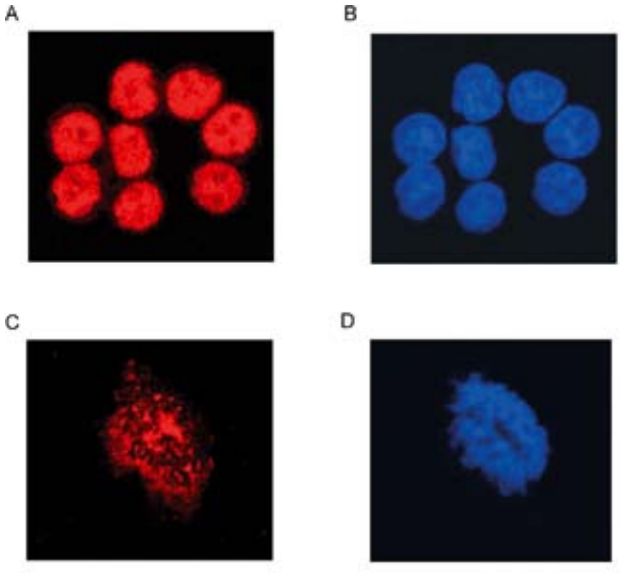

Figure 1
Intracellular localisation of TopBP1 during G1/S and M phases (A, C) of the cell cycle. HeLa cells were fixed and stained with anti-TopBP1 (A, C, red) and Dapi (B, D, blue). Support by G. D. Wieland (HKI Jena) is kindly acknowledged.

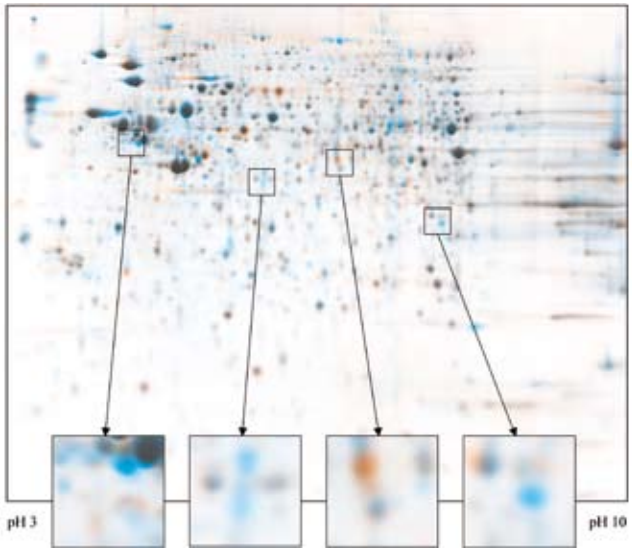

Figure 2
2-dimensional separation of the proteome of HepG2 cells infected with Coxsackievirus B3 and uninfected HepG2 cells. Upregulated protein spots of infected cells appears blue whereas downregulated spots are orange.

ribosylation of PARP-1 and is ADP-ribosylated by PARP-1. The complex formation between TopBP1, PARP-1 and Miz-1 (and other proteins) and the disruption of these complexes under different conditions, e.g. DNA damage, raise the question whether TopBP1 acts as a scaffold protein that binds or releases proteins under varying physiological conditions.

In parallel, we performed a detailed analysis of the TopBP1 promoter region and showed that Egr-1 stimulates a TopBP1 promoter-luciferase construct. The transcription factor early growth response 1 (*Egr1*) belongs to a group of early response genes transiently induced by several environmental signals such as growth factors, hormones, UV light, stress and hypoxia. A very recent report showed involvement of Egr-1 in a signaling cascade involving sequential activation of the nuclear factor κB (NF-κB), Egr-1 and the growth arrest and DNA damage inducible gene 45 (Gadd45) to induce UVB-mediated cell death, a process which is also regulated by the human topoisomerase IIβ binding protein 1 (TopBP1; Thyss et al., 2005). Performing EMSA and ChIP experiments we showed that the Egr-1 protein binds specifically to an oligonucleotide spanning the hypothetic Egr-1 binding site near the transcriptional initiation site of the TopBP1 gene *in vitro* and *in vivo*. From the analysis of nested 5´deletions of the TopBP1 promoter fragment followed that deletion or mutation of one E2F transcription factor binding site near the Egr-1 binding site leads to a declined stimulation of the TopBP1 promoter by Egr-1. This indicates cooperative effects between Egr-1 and E2F in transactivating the TopBP1 promoter.

Recent studies of several groups revealed that TopBP1 is a potential therapeutic target for cancer treatment. Downregulation of TopBP1 protein level in human cells by siRNA-approaches induced apoptosis due to derepression of the pro-apoptotic factor E2F-1. Thus, cells harboring higher E2F-1 levels would be more susceptible to inhibitors of TopBP1. Most cancer cells contain excessive E2F-1 activities because of de-

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regulation in the Rb pathway. Thus, TopBP1 is a potential therapeutic target to harness E2F-1 for cancer treatment. (Figure 1)

5 Host Cell Response to Pathogenic Infections

Group Leader: Thomas Munder

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 at 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 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 NFκB 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. (Figure 2)

6 Transcriptome-based Analysis of Cellular Response to Natural Products

Group Leader: Hans-Joachim Krügel

Host Cell Response, Natural Products

Within this HKI network project we investigated host response to peptaibol treatment in the context of psychotic disorders. Psychotic disorders which affect up to 1% of the human population (Bray & Owen, 2001) represent pathological changes to the metabolic homeo-

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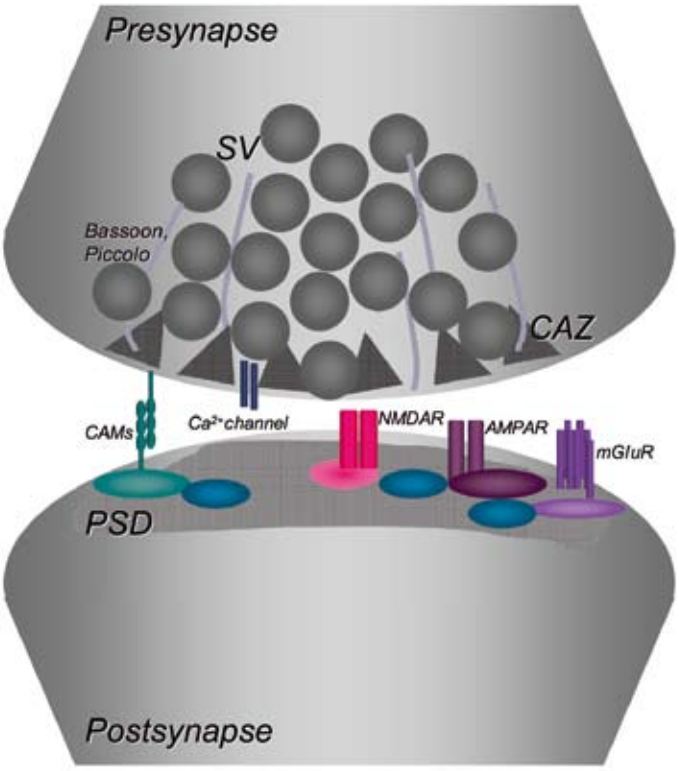
Figure 3
Ampullosporin induced reorganisation of synapses. Cells in the frontal cortex respond to Ampullosporin by inducing massive changes in neurotransmitter receptor composition, in the calcium signaling cascades, and the second messenger systems, leading to the plastic reorganisation of brain tissue, metabolic pathways and synapses. SV - Synaptic Vesicle, CAZ - Cytomatrix at the Active Zone, CAMs - Cell Adhesion Molecules, mGluR - Metabotropic Glutamate Receptor, AMPAR - AMPA-type Glutamate Receptor, NMDAR - NMDA-type Glutamate Receptor, PSD - Postsynaptic Density



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stasis of the brain. We focused on the analysis of molecular events occurring in the brain of ketamine-pretreated rats after administration of Ampullosporin A with neuroleptic-like activity. The complex experimental approach allowed correlating the use of low molecular weight substances with a transcriptome fingerprint in the prefrontal cortex. We identified 63 genes up-regulated and 22 genes suppressed with transthyretin, syndecan-1 and NeuroD1 showing the highest degree of up-regulation. Our results raise the possibility that Ampullosporin A belongs to the group of neuroleptic-like compounds, inducing massive changes in neurotransmitter receptor composition, calcium signalling cascades, and second messenger systems, leading to the plastic reorganisation of brain tissue, metabolic pathways and synapses.

Transcriptome based methods were applied to the molecular characterization of newly identified compounds with interesting pharmacological profile. Many pharmacological activities may be accompanied by cellular reorganisation, preceded by transcriptional activities responding to the disequilibration caused by the drug. Very complex biochemical processes are involved in the stabilization of mood and behaviour by neuroleptics. An animal model uses ketamine simulating the schizophrenic situation. Administration of neuroleptics allows the investigation of their biochemical and physiological activities.

One example represents peptaibols, peptides of fungal origin with diverse biological activities. From a broad range of peptaibols Ampullosporin A is the best-characterized compound with respect to its specific biological activity. Most peptaibols are known to form ion channels or pores in biological membranes. These activities are correlated to their length and hydrophobicity/hydrophilicity and charge distribution. Ampullosporin contains three glutamine residues which are located on one side of the amphipathic helix. This may be

the basis for either a specific pore formation depending on the membrane potential or a very specific interaction with the ligand-binding portion of certain receptors, e.g. the NMDA receptor.

A very surprising observation was the occurrence of melanin formation by the fungus *Phoma destructiva* in response to treatment with cyclosporine. This effect is to a much lesser degree, but strongly correlated, with all known neuroleptic drugs. The rat model consists of acute response analysis in a rod-jump and social behaviour tests of rats pretreated with ketamine after administering of Ampullosporine. Other peptaibols which show the "Phoma-effect" remain to be tested with respect to their neuroleptic activity in the rat model. Mice hypothermia might be misleading as far as correlated with undesirable side effects. Increasing evidence in the literature suggests complex biochemical and/or transcriptional alterations accompanying schizophrenia-like phenomena. Sub chronic treatment with subanesthetic doses of ketamine induces schizophrenia-related psychotic alterations (Lahti et al., 1995), which can be used as an animal model in the study of this disorder (Becker et al., 2003). Ampullosporin A belongs to a specific group of pore-forming fungal peptides, peptaibols (Kronen M. et al., 2003). We isolated RNA from rat brain treated with ketamine and after the administration of ampullosporine A and used labelled c-RNA for the hybridization of a 20k rat oligonucleotide array (Agilent) and observed changes in the transcription pattern of genes in the *cortex prefrontale*. The experimental approach allowed to correlate the use of a new fungal product with a transcriptome fingerprint in the rat brain preconditioned by ketamine. Our focus was on those genes, which were affected only in cells responding to AmpA when pretreted by ketamine, but not in the control cells solely treated with AmpA, ketamine, or salt solution. Within the 63 genes up- regulated and 22 genes suppressed we found transthyretin, syndecan-1 and NeuroD1 showing the highest degree of upregulation. Our results reveal the cellular response to a natural compound by inducing massive changes in neurotransmitter receptor composition, in the calcium signalling cascades, and the second messenger systems, leading to the plastic reorganisation of brain tissue, metabolic pathways and synapses. Moreover, our data strengthened importance of natural compounds as research tools for the understanding of complex cellular responses to perturbations of the cellular equilibrium. (Figure 3)

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

Group Leader: Alexander Tretiakov

SpeedCycler

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 microfabricated 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 pairwise 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 fol-

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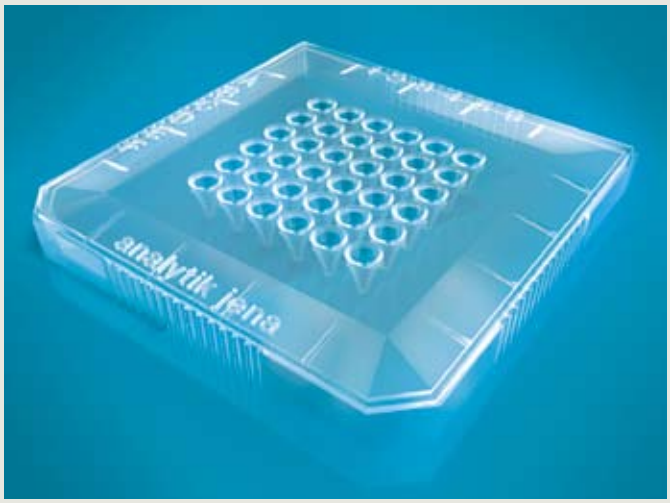

Figure 4
Rapid heat block thermocycler (SpeedCycler, Analytik Jena) and ultra-thin walled microwell plate.

lowing 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 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. (Figure 4)

8 Development of Rapid and Sensitive Methods for Pathogen Detection in Shrimp

Group Leader: Hans Peter Saluz

SpeedCycler

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.

9 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. 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.
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.

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Department of Molecular Natural Products Research

Department of Molecular Natural Products Research

The Department of Molecular Natural Products Research focuses on strategies directed towards the exploitation of structural diversity from natural sources with the aim of providing new compounds for biological characterization. Our strategies are chosen with respect to the discovery of new lead compounds for drug development. However, we also supply small molecules from natural sources as tools for studying biological communication processes.
Compounds from microbial cultures are the predominant source for drug discovery from natural sources. Namely, sporoactinomycetes and fungi with their unique potential of biosynthetic pathways are in the focus of our efforts. Supplementing genetic engineering based approaches of other HKI groups we address turning on and off biosynthetic pathways by variation of culture conditions or media to modify the variety of secondary metabolites. Picking the "right" compound is what we consider as a key issue of our natural products chemistry activities. For this purpose, we combine chemical and physico-chemical analysis (automated and standardized thinlayer chromatography, HPLC-UV, HPLC-MS, HPLC-MS^n) that also contribute to the generation of in-house databases with results from biological testing achieved by external collaboration and within the HKI Internal Product Line (IPL). We consider the elucidation of the mode of action of secondary metabolites as a most interesting aspect of drug directed natural products research. Therefore, in cooperation with Friedrich Lottspeich at the Max

INTRODUCTION | EINLEITUNG

Head:
Prof. Dr. Susanne Grabley

Das wissenschaftliche Anliegen der Abteilung Molekulare Naturstoff-Forschung ist die Erschließung und Nutzung struktureller Diversität aus der Natur. Dabei ist das vorrangige Ziel, neue niedermolekulare Verbindungen aus Mikroorganismen zugänglich zu machen und in Kooperationen ihre biologische Aktivität zu evaluieren und zu profilieren. Mit neuen Methoden und Techniken der Proteomanalyse, wie sie in der Arbeitsgruppe von Friedrich Lottspeich am Max-Planck-Institut für Biochemie, Martinsried, entwickelt werden, eröffnen sich für uns neue Wege, das Interaktionsnetzwerk von Natur- und Wirkstoffen zu visualisieren, zu verstehen und für die Wirkstoffoptimierung zu nutzen. Darüber hinaus stellen wir niedermolekulare Naturstoffe als Tools für die molekular- und zellbiologische Forschung zur Verfügung. In das HKI-interne Netzwerk Grundlagenforschung war die Abteilung im Berichtszeitraum 2004 mit insgesamt vier Projekten eingebunden.

Unter den Mikroorganismen zeichnen sich insbesondere Sporoactinomyceten und Pilze durch ein hohes Potential an Stoffwechselwegen aus, die zu einer einzigartigen Vielfalt an niedermolekularen Naturstoffen führen. In Ergänzung zu anderen HKI-Arbeitsgruppen stehen neben der Entwicklung von Methoden und Strategien zur Detektion und Kultivierung neuer Isolate nicht molekulargenetische Methoden, sondern Parameter wie Anzuchtbedingungen und Nährmedien als Werkzeuge zum An- und Ausschalten einzelner Stoffwechselwege und damit zur Einflussnahme auf das Produktspektrum im Vordergrund der Aktivitäten.
Ausgangspunkt und Herzstück der naturstoffchemischen Arbeiten ist die chemische und physikochemische Analytik (automatisierte

Planck Institute for Biochemistry (Martinsried) we supply drug tested cell cultures for proteome analysis with the aim to visualize, understand and modify the interaction network of bioactive compounds in the cellular environment. As a prerequisite, we have established appropriate cell culture techniques together with the HKI Department of Drug Testing.

In order to realize our concepts and strategies we have built a strong project network with internal and external partners from academia and industry as well. Within joint projects funded by the BMBF we succeeded in optimizing our approaches for natural product isolation and rapid identification. Furthermore, we have been able to extend our spectrum of natural sources, substantially: Within the BMBF supported project "Drug Discovery with Fungi from Marine Habitats" which came to a first end in Summer 2002 and has been continued in 2004 we are investigating fungal isolates from cold marine environments. So far, 17 new natural compounds showing either differential cytotoxicity or EGF receptor activity have been claimed in a comprehensive patent application by our industrial partner BRAIN AG. Within the framework of the BMBF supported Chinese-German project "EXTENSION" with Yunnan University we have discovered new highly potent members of the griseusin and the gilvocarcin family originating from alkaliphilic and halophilic actinomycete isolates from unusual habitats in China. Their cytotoxic properties encouraged our industrial partner

und standardisierte dünnschichtchromatographische Analytik, HPLC-UV, HPLC-MS, HPLC-MS^n). Ergänzt werden die abteilungsintern gewonnenen und über ein Datenbank-Managementsystem aufbereiteten Ergebnisse durch Kooperationen zur Auffindung und Charakterisierung biologischer Wirksamkeiten; HKI-intern spielt dabei die Durchgehende Bearbeitungslinie (DBL) eine besondere Rolle.

Die Abteilung sieht es als eine ihrer Hauptaufgaben an, zur Aufklärung der Wirkungsweisen von Naturstoffen beizutragen. Methoden und Techniken der funktionalen Proteom-Analyse eröffnen neue Wege, das Interaktionsnetzwerk von Wirkstoffen aufzudecken und für die Wirkstoff-Optimierung zu nutzen (in Zusammenarbeit mit Friedrich Lottspeich, Max-Planck-Institut für Biochemie, Martinsried). Wir haben daher die im Jahr 2002 begonnen Arbeiten zur Etablierung und Nutzung geeigneter Zellkultur-Systeme für die Proteom-Analyse in Kooperation mit externen Partnern fortgesetzt.

Im Rahmen projektbezogener Arbeiten mit Kooperationspartnern in Deutschland, im europäischen Ausland und zunehmend auch in der V.R. China konnten wir die Basis für unsere Naturstoff-Isolierungsstrategien erweitern und das Spektrum an Naturstoff-Quellen ergänzen: Als Ergebnis einer ersten dreijährigen Förderphase wurden im Rahmen des BMBF-Verbundprojektes „Wirkstoffe aus marinen Pilzen", das insbesondere auf die Identifizierung neuer Tumortherapeutika abzielt, 17 neue Naturstoffe von unserem Partner BRAIN AG zum Patent angemeldet. Die Arbeiten wurden Mitte 2004 nach fast zweijähriger Unterbrechung in einer zweiten Förderphase fortgesetzt. Aus unserer Zusammenarbeit mit der Oncotest GmbH im deutsch-chinesischen BMBF-Verbundprojekt „EXTENSION" wurden aus Actinomyceten

Oncotest to file a patent on their use as new potential anti-cancer drugs. Due to extended cooperation with Chinese partners in Beijing engaged in the investigation of medicinal plants we stepped into the field of isolating and cultivating plant associated microorganisms with a focus on sporoactinomycetes and filamentous fungi. Funded by the BMBF, in the "MONACO" framework we got access to fresh material of a series of mangrove species from the coast areas of Xia Men and Hai Nan. Until May 2005, two PhD students from Peking University have graduated after stays in Jena for more than 18 months. Their studies yielded 56 new natural compounds that in parts are still under investigation with respect to their biological activities. Within the IPL we have been able to discover and characterize four new secondary metabolites which we called lydiamycins. They are unique representatives of the cyclodepsipeptide family with inhibitory effects against mycobacteria.

The Natural Products Pool with about 9.000 compounds from more than 90 groups from academia worldwide has been used in industrial drug discovery for more than nine years. Already during this period of industrial financing, ten academic groups had access to the Natural Products Pool for their biological testing interests. However, in the future the Natural Products Pool is supposed to serve as an important tool for academic screening purposes, possibly within the framework of the national "ChemBioNet" initiative, e.g. in the field of chemical genomics.

extremer Habitate der V.R. China (neben mehr als 15 weiteren neuen Naturstoffen) neue hochpotente Vertreter der Griseusine und Gilvocarcine gefunden, die als potenzielle Krebstherapeutika zum Patent angemeldet wurden. Unser zweites deutsch-chinesisches BMBF-Verbundprojekt „MONACO", in dem Mangroven südchinesischer Küstenregionen und ihre Mikrobionten untersucht werden, führte zum sehr erfolgreichen Abschluss von zwei ersten gemeinsam betreuten Dissertationen von Doktoranden der Peking University mit der Charakterisierung von insgesamt 56 neuen Naturstoffen. Im Rahmen der DBL konnten mit den Lydiamycinen vier neue strukturell einzigartige Vertreter cyclischer Depsipeptide mit Wirkung gegen Mykobakterien zum Patent angemeldet werden. Mit dem Naturstoff-Pool Projekt, an dem weltweit etwa 90 akademische Arbeitsgruppen als Zulieferer beteiligt sind, sind wir nach mehr als neun Jahren erfolgreicher industriefinanzierter Arbeit in eine Umbruchphase gekommen. Schon bisher konnten zehn akademische Arbeitsgruppen auf den Naturstoff-Pool zur biologischen Testung zurückgreifen. Im Rahmen neuer bilateraler akademischer Kooperationen und der neuen nationalen Initiative „ChemBioNet" zu Chemical Genomics steht nun die Entwicklung neuer Konzepte an.

Scientific Projects

1 Natural Products Discovery

Group Leader: Isabel Sattler

(on leave from May 2004 till February 2005, from July till December 2005 as independent research group)

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

Abstract

Considering the ongoing importance of natural products as lead compounds or blueprints in the search for bioactive principles, it is our major interest to identify new substances from microbial sources, with a major focus on actinomycetes or filamentous fungi. Our expertise includes cultivation of microorganisms, standardized and automated extract preparation by solid phase extraction, physico-chemical analysis of extracts, and most importantly, chromatographic preparation of pure compounds from large scale cultivation (by HKI pilot plant) and structure elucidation. Thus, our work includes all methods of modern natural products chemistry, in particular mass spectrometry and NMR spectroscopy, as well as tools of stereochemical analysis, e.g. CD spectroscopy and chemical derivatization.

In the search for interesting extracts, and thus promising producing microorganisms, we efficiently interwine biologically driven approaches, e.g. antimicrobial and cytotoxicity screening, with the physico-chemical analysis of the complex compound mixtures. For biological testing, we rely on our colleagues of the IPL team and external collaboration partners. Depending on the individual project objectives, we can start from either end, always following the major goal to access meaningful, and best, structurally new compounds in the subsequent chemical projects.

Screening Programs

Structural originality and diversity is assumed to be closely related to the quality of the biological sources that are used. 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

(i) extremophilic and rare actinomycetes, as well as filamentous fungi, from unusual habitats from the southwest of China (provided by Yunnan Institute of Microbiology, 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, Universität Jena)

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. Thus, we are currently working on bridging the gap between the pure sceening routine, that typically involves strain cultivation in shaking flask cultures, and the subsequent preparative process, that normally relies on strain cultivation in fermentors up to 300 L. This dramatic difference in growth parameters, very often affects the secondary metabolite pattern in an unpredictable manner. This problem is particulary crucial, as

Table 1
Antibacterial activities of lydiamycins A-C (**1**-**3**)

[a] mean of three independent experiments.

Organism	MIC/µg mL^{-1} [a]		
	1	**2**	**3**
Bacillus subtilis ATCC 6633	100.1	100.1	100.1
Mycobacterium smegmatis SG 987	25.0	25.0	25.0
Mycobacterium aurum SB66	6.3	6.3	12.5
Mycobacterium vaccae IMET 10670	3.1	3.1	3.1
Mycobacterium fortuitum B.	100.1	100.1	100.1

Table 2
Activity of lydiamycin A (**1**) against *M. tuberculosis* strains

[a] minimal bactericidal concentration

[b] clinical isolate resistant against main antituberculosis drugs

[c] isoniazid

strains	**compounds**	**MIC/µg mL^{-1}**	**MBC[a]/µg mL^{-1}**
H37Rv	lydiamycin A (**1**)	12.5	25
# 246[b]		25	50
H37Rv	control[c]	1.17	1.56
# 246		>100	>100

our general research objective only allows to a very limited extent to study invidual strains in greater detail. Thus, we are setting up a second round of advanced screening in a growth scale, e.g. 5 L, that still allows some degree of parallelization and the preparation of extracts in a regular lab routine, but on the other side enables access to pure compounds on a low mg-scale. This semipreparative approach enables the reliable identification of the true active principles in the extract mixtures. The application of HPLC/MS-driven separation on the analytical and preparative scale with a high degree of standardization is supposed to readily identify known from potentially new compounds. This modified approach is now consequently pursued in a second project phase of our work on marine fungi from cold marine habits, where from a set of 606 fungal isolates, 68 isolates are now studied in greater detail.

Another focus on advancing our screening routines is set on improving dereplication procedures for extract screening by applying HPLC/MS^n-analysis. After determining suitable parameters for MS detection of a broad range of structurally diverse compounds, we have substantially proceeded in setting up a library with natural products that up till now contains four thousand compounds.

Lydiamycins A-D: a New Class of Small Cyclodepsipetides with Anti-Mycobacterial Properties

In the course of our investigations of natural products from microorganisms for drug discovery, we studied *Streptomyces lydicus* (strain HKI0343) by physico-chemical metabolite pattern analysis and thus discovered four new cyclodepsipeptides, lydiamycin A-D (**1**-**4**). The structure determination was achieved using a combination of CID ESIMS/MS data and 1D and 2D NMR techniques, such as DEPT, COSY, HMBC, TOCSY and ROESY, the assignment of the absolute configurations, and, finally,

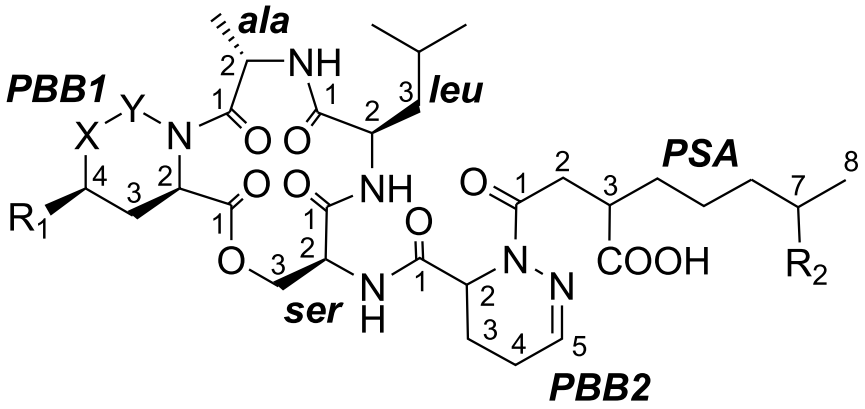


Figure 1
Structures of lydiamycins A-D (**1-4**)

	R_1	R_2	X-Y
1	H	H	$-CH_2-NH-$
2	OH	H	$-CH_2-NH-$
3	H	H	-CH=N-
4	H	OH	$-CH_2-NH-$

biological properties. For determination of the absolute configurations, an acidic hydrolysate generated according to the Marfey protocol, and subsequent HPLC analysis indicated the presence of L-alanine, D-leucine and L-serine. The stereochemistry of the piperazic acid-moiety PBB1 was determined through conformational analysis and MM+ force field computation. Thus, the 2*R* configuration was suggested for PBB1. The absolute configuration at C-2 of PBB2 has to remain unassigned.

In our biological profiling program of new natural products identified by physico-chemical screening, lydiamycins A – C (**1 – 3**) were shown to selectively inhibit *Mycobacterium smegmatis* SG 987, M. *aurum* SB66 and *M. vaccae* IMET 10670 within a panel of Gram-positive and Gram-negative bacteria, yeasts and fungi. (Table 1) No cytotoxicity was found against L-929, K562, HeLa, MDCK and GMK cell lines. Further studies on the antibiotic activity of lydiamycin A against slow growing and pathogenic mycobacteria, including the *M. tuberculosis* standard strain H37Rv and a multiresistant clinical isolate, confirmed its anti-mycobacterial properties. (Table 2) Interestingly, activity against the multiresistant strain of *M. tuberculosis* suggests a mechanism of action different to that of available therapeutics like isoniazid, rifampicine, etambutol and streptomycin.

From the structural point of view, the lydiamycins represent a novel class of small cyclodepsipeptides. Next to their small ring size, the most outstanding structural feature of the lydiamycins is the presence of the piperazic acid building blocks which are not found in any of the other small congeners. However, two piperazic acid building blocks are the major characteristic of the nineteen-membered cyclodepsipeptides of the azinothricin family, e.g. aurantimycins and verucopeptin. A common feature of the azinothricin family and some other cyclodepsipeptides, as well as with the lydiamycins, is the presence of one polyketide chain of varying complexity and length.

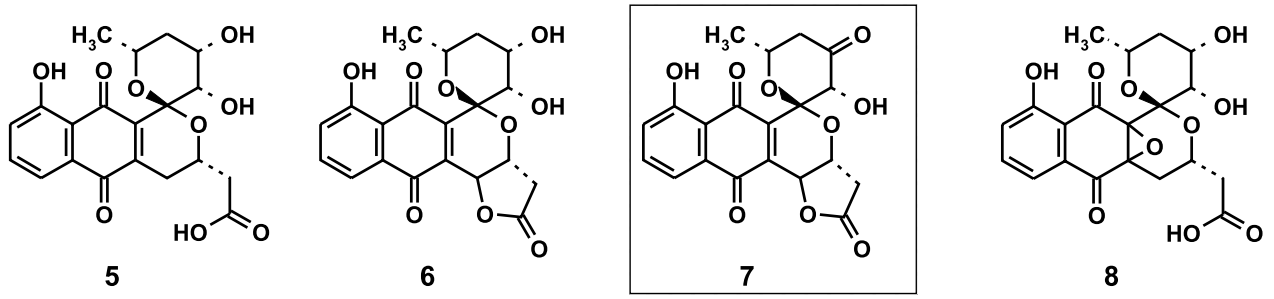
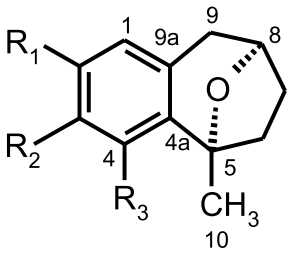


Figure 2
Structures of epi-deacetylgriseusin B (5), epi-deacetylgriseusin A (6), 4'-dehydro-deacetylgriseusin A (7) and 2a,8a-epoxy-deacetylgriseusin B (8).

Figure 3
Structures of bruguierols A-C (9-11)



	R_1	R_2	R_3
9	OH	H	H
10	OH	OH	H
11	OH	H	OH

New Cytotoxic Griseusin Derivatives from *Nocardiopsis* spec.

Within the framework of the BMBF supported Sino-German joint project "EXTENSION" ("Extreme and Unusual Environments as Sources for New Drugs from Microbial Origin") our responsibility covered natural products microbiology for 305 isolates from unusual habitats in China provided by our academic partner from Yunnan University, P.R. China. Our German industrial partner Oncotest GmbH Freiburg performed an initial cytotoxicity screening on six tumor cell lines that differed in chemosensitivity towards standard chemotherapeutic agents.

A group of four griseusins from *Nocardiopsis* sp. DSM16644 with two new derivatives, 4'-dehydro-deacetylgriseusin A (**7**) und 6,7-epoxy-deacetylgriseusin B (**8**) was most noteworthy. 4'-Dehydro-deacetylgriseusin A showed a very high antitumor potency (mean IC_{50} = 0.172 µg/mL) combined with a significant selectivity for mammary cancer, renal cancer and melanoma in a cell line panel consisting of 36 tumor cell lines reflecting 14 different solid tumor types. As **7** was only isolated as minor component, studies for semisynthetic access were performed. Among various oxidation reactions tested, only the Jones reagent (chromate sulfuric acid in acetone) allowed a smooth synthesis from epi-deacetylgriseusin A (**6**). Our studies also yielded new synthetic derivatives of those isochromanquinones. A preliminary SAR study clearly showed that the 4'-keto group is essential for the strong cytotoxic activity, that was not known for this class of compounds before. Surprisingly, the oxirane functionality is detrimental to the cytotoxic activity.

Plant Metabolites

During the last decades it was shown that higher plants host a tremendous diversity of endophytic microorganisms, many of them being actinomycetes and fungi. As they are readily available to cultivation, they have

attracted major interest, in particular for metabolites that originally were supposed to be of mere plant origin. In a joint research project with Peking University, we are studying mangroves and their associated microorganisms for their natural products. We have chosen mangroves as model system, as those marine plants represent an unique ecological system, which can be found on tropical and sub-tropical coast lines. Due to tidal changes they have to deal with regular and drastic change of conditions (salt exposure, nutrient supply), and through the necessary adaptations processes they are considered to be a rich source of secondary metabolites. Some mangroves are used in folk medicine (e.g. Traditional Chinese Medicine) and thus are considered a potential source for new compounds in drug discovery. Within our project samples of 14 mangrove species have been collected from two sampling sites on the south coast of China and have yielded 135 fungi and 105 actinomycetes.

In the phytochemical investigations of this project, the stem of *Bruguiera gymnorrhiza* yielded among other compounds, three new aromatic compounds called bruguierols A-C (**9 – 11**) which represent a new structural skeleton of secondary metabolites. In our biological profiling program, **11** was found to have moderate activity against Gram-positive and Gram-negative bacteria including one mycobacterial strain and the resistant *Enterococcus faecalis* 1528 (VanA) with a mean MIC of 12.5 µg/mL.

Natural Products Pool

The academic application of the HKI Natural Products Pool enabled the identification of the known fungal metabolite chaetocin as a first specific inhibitor of a lysine-specific histone methyltransferase. The post-translational modification of histones plays a key role in regulating gene expression and thus is considered a potential target for cancer therapy. The *in vitro* inhibition of the *Drosophila melanogaster* SU(VAR)3-9 protein in the screening assay (sub-micromolar concentration) was also confirmed in a cell culture model. These results, and also those from other screening programs (not shown), impressively demonstrate the importance of the availability of a standardized collection of natural products for the academic screening community. The advance of novel assay systems, many of them only becoming possible through recent genomics approaches, thus, allows a renaissance of long known compounds.

Recent calculations by the Computer-Chemistry-Center at the University of Erlangen-Nürnberg (Prof. Gasteiger, personal communication) in a self-organizing neural network approach with 27 dimensional autocorrelational vectors as structural descriptors attest the HKI Natural Products Pool a remarkably broad structural diversity (Figure 1). Although the HKI Natural Products Pool with about 8.700 compounds is a rather small library as compared to regular HTS-libraries, it showed a diversity similar to that of WOMBAT (Sunset Molecular Discovery LLC, U.S.A.), a large dataset with nearly 76.000 known ligands for a vast variety of enzymes. This comparison also revealed several overlapping clusters that confirm "ligand-likeness" of the natural products which can readily be explained by their evolutionary background. (Figure 4)

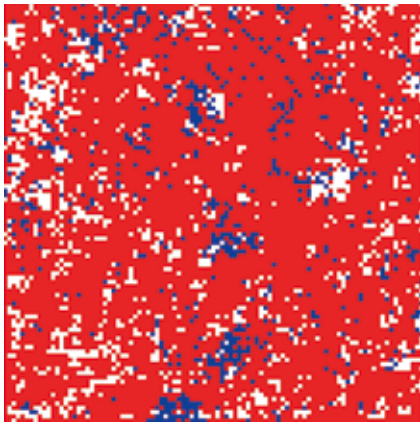


Figure 4
Schematic representation of the structural diversity of the HKI Natural Products Pool (blue dots) in comparison to the WOMBAT dataset (red dots), as calculated by a self-organizing neural network (total 83 862 compounds, 100 x 100 neurons) (courtesy of Professor Gasteiger prior to publication)

Summary

Taking all activities together, in 2004 and 2005, more than 5600 microbial extracts from 872 different strains have been studied within the various screening programs. Subsequently, 31 strains were studied in greater detail, of which 33 300 L cultivations have been performed by the HKI Department of Pilot Plant for Natural Products. Of the many compounds, obtained after downstream processing and natural products chemistry, more than 65 significant secondary metabolites have been structurally assigned. 20 of these compounds could not be found in the literature. In addition, more 23 compounds have been isolated from plant material. Altogether 107 samples have been provided to the HKI Internal Products Line (IPL) showing various biological effects that are currently studied in more detail.

2 Taxonomy (2004)

Group Leader: Ingrid Groth

Search for Novel Bioactive Secondary Metabolites in Actinomycetes and Fungi

Abstract

Environmental microbial isolates from unusual habitats are considered as a promising resource of novel chemical compounds of pharmaceutical significance.Within the frame of a project funded by the EU numerous actinomycete isolates from unexplored hypogean environments were obtained. A preliminary phenotypic characterization of these isolates contributed to the recognition of strains belonging to genera which are already known as producers of commercially significant bioactive secondary metabolites. The strains of these genera were included in the primary screening for novel natural compounds. Furthermore detailed taxonomic studies of unusual isolates of the genus *Kitasatospora* were performed which revealed the existence of novel species. The description of these novel species was published in the *International Journal of Systematic and Evolutionary Microbiology*.

Within the frame of the EU project "Cyanobacteria attack rocks: Control and preventive strategies to avoid damage caused by cyanobacteria and associated microorganisms in Roman hypogean monuments" (EVK4-CT-2000-00028) we analysed the occurrence of heterotrophic bacteria in cyanobacteria dominated biofilms in Roman catacombs and in the Cave of bats (Zuheros, Spain). From about 900 bacterial isolates more than 700 strains were found to be members of the order *Actinomycetales*. These strains were phenotypically characterized and assigned to 27 genera of this order. A high amount of the strains could be affiliated to the genus *Streptomyces* and to genera of the so called "Rare actinomycetes". Strains of these genera are already known as producers of interesting natural products. 287 selected isolates were cultivated under laboratory conditions in four different culture media. As members of the genus *Streptomyces* are generally well studied concerning their secondary metabolites, we included only those strains in the screening which were resistant to the polyvalent *Streptomyces* phage S7. It was found that this phage lyses about 20% of *Streptomyces* strains obtained from terrestrial samples and that the sensitive strains belong to different species. In contrast, the amount of S7-phage resistant strains derived from hypogean environments was in the range of 40–50%. But in some sampling sites up to 75% of the *Streptomyces* isolates were resistant to this phage. Therefore the resistance to phage S7 indicated that a special streptomycete population occurred in these habitats.

Extracts of the culture filtrates and the biomasses of the selected isolates were checked for bioactivity in the test systems of the Internal Product Line of the HKI. Additionally the extracts were tested in the thinlayer chromatography based chemical screening of our department.

The results of the biological screening are given in table 3. They show that about 70% of the tested isolates were able to produce bioactive compounds under the applied laboratory conditions. Of special interest were those isolates which showed very selective activities against special test organisms, for instance against *Candida albicans*. This was the case in 9 isolates belonging to the genera *Streptomyces* (6), *Nocardioides* (1), *Promicromonospora* (1) and *Pseudonocardia* (1). The results of the chemical screening similarly indicated that in about 60 % of the tested extracts interesting compounds were detected on the chromatograms.

The results of the primary screening convincingly demonstrated that actinomycetes adapted to the special conditions of hypogean environments are able to produce bioactive compounds. However further studies have to be performed to clarify if the bioactivities found in the crude extracts are related to novel chemical structures.

Further activities of our group were related to the laboratory cultivation of about 25 actinomycete and 70 fungal isolates within the frame of the primary screening for novel compounds. These strains were obtained from the former alum slate mine Feengrotten (Saalfeld), a very

Taxon	Results	Bioactivity				
	Number of strains	**Number of positive strains**	**R9**	**H8**	**B4**	**M2**
Agromyces sp.	21	14	3	1	-	14
Amycolatopsis sp.	8	5	1	1	1	5
Micromonospora sp.	6	6	2	-	-	6
Nocardioides sp.	17	8	2	1	1	5
Nocardiopsis sp.	6	3	2	2	1	1
Oerskovia sp./ *Promicromonospora* sp.	26	21	6	9	4	17
Pseudonocardia sp.	2	1	-	1	-	-
Saccharopolyspora sp.	1	-	-	0	-	-
Saccharothrix sp.	15	10	6	4	1	7
Streptomyces sp.; S7 negativ	185	134	84	23	35	94

Testorganisms:

Escherichia coli SG 458	B4
Mycobacterium smegmatis SG 987	M2
Candida albicans BMSY 212	H8
Staphylococcus aureus/MRSA + Chinolon-r 134/93	R9

Table 3
Biological activities of actinomycete isolates from hypogean enviroments

acidic and heavy metal containing hypogean biotope. Similarily to the isolates from the Roman catacombs a high number of the tested actinomycetes and fungi from this habitat was able to produce bioactive compounds.

In collaboration with the group of Prof. E. Kothe (FSU) interesting heavy metal resistant actinomycetes from the uranium mining area around Ronneburg, Thuringia, were cultivated under different stress conditions in our laboratory to check their potential for the production of bioactive compounds in the primary screening of the HKI.

Similarily in collaboration with the Fungal Reference Center of the FSU (group of Dr. K. Voigt) 80 different fungal strains were cultivated in the search for novel fungal secondary metabolites.

As the secondary metabolites of novel taxa are of special interest, the identification of unusual isolates is of great importance. Therefore in collaboration with the group of M. Goodfellow (Newcastle, UK) a comprehensive polyphasic taxonomic study was performed to establish the taxonomic position of actinomycete isolates which were affiliated to the rare genus *Kitasatospora*. Based on about 20 preselected strains from different geographic regions five novel species of the genus *Kitasatospora* were published.

3 **Molecular Proteomics** (2004)

Group Leader: Thomas Munder

HepG2 Cell Cultures as a Model System for Proteome Analysis of Cytotoxic Natural Compounds

Abstract

Natural products contribute substantially to the discovery of new therapeutic principles and new therapeutic targets. Furthermore, about 30% of the drugs on the worldwide market are natural products or are derived from natural

products. A similar ratio accounts for clinical candidates currently under development. Considering only anti-cancer drugs, natural products are of even more importance. However, although in most cases the main mode of action is well documented new approaches are requested to understand the spectrum of side effects often resulting from touching a complex network of cell signalling pathways. Functional proteomics gives rise to a quantitative assessment of the entire protein network which is an extremely complex and highly dynamic system characterized by e.g. variation of protein expression, feed back regulation or protein isoform formation as well as numerous posttranslational modifications, degradation and trafficking that lead to protein activation or inactivation.

Functional proteomics with respect to drug profiling is only at the beginning. It is supposed that about 30,000 to 50,000 different protein species have to be considered at a given state. Approaches that capture only parts of the entire network and that do not deliver simultaneous quantitative comparisons will lead to inferior and uncompleted drug solutions. So far, a number of key issues still have to be improved such as detection limits (copies of proteins per cell), detection ranges (highly expressed proteins beside minor ones; the dynamic range accounts for more than 10^4), coverage of protein species, protein sequence coverage as well as reliability and reproducibility of measurements and their interpretation by means of imaging techniques and bioinformatics. As a most promising development towards a quantitative registration and identification of the entire proteome the so-called ICPL technology has to be considered. Within the BMBF supported joint project "MONACO" (High Throughput Monitoring for Functional Analysis of Natural Compounds) started in April 2003, we get access to this completely new technique in proteome analysis which is capable of handling all kinds of proteins within a large dynamic range and with high sensitivity. In cooperation with the group of F. Lottspeich, MPI for Biochemistry, Martinsried, we address a comprehensive analysis of the bioactive properties of cytotoxic natural compounds. As a first step and with the support of the HKI Department of Drug Testing we have set up an appropriate cell culture regime that in a second step has to be validated and evaluated with a selection of standard compounds. Our medium and long term interest is to characterize new compounds with potential in anti-cancer therapy arising from our screening programs towards drug discovery from nature, and to eventually identify new targets for anti-cancer therapy. To characterize and to optimize our model system, the human hepatocellular carcinoma cell line HepG2, a number of biochemical and cell culture experiments has been performed accompanied by microscopic monitoring of cell shape, viability and further parameters. Most crucial for the interpretation of drug testing data are individual threshold values that refer to cell viability and induction of cell death, e.g. the apoptotic cascade (Vaux and Korsmeyer, 1999). These drug concentrations are taken as the basis for our "large-scale" cultivation processes (300 cm^2 containing about 6 x 10^7 cells when harvested) to analyze the drug effects on the protein composition of the HepG2 cells by ICPL-technique at Prof. Dr. F. Lottspeich's group in Martinsried. However, in order to check, control and standardize our cultivation parameters and drug testing protocols it was necessary to set up a 2D gel electrophoresis system in our lab as well. In close collaboration, several protocols have been tested and modified to find an optimal standard procedure for the test sample preparation of the HepG2 cell extracts. In order to standardize and to evaluate our system five compounds with known cytotoxic effects, adriamycin, camptothecin, cyclosporin A, paclitaxel and trichostatin A, have been selected for detailed studies. Various methods for the determination of cytotoxicity and anti-proliferative activity as for apoptotic activity have been addressed (Dahse et al., 2001). In our hands the technique of staining cells with methylene blue in combination with a cell viability counter is the most effective method to access first compound specific concentration related parameters. In our standard operation procedure the standard compounds are added to growing HepG2 cells in their individual threshold concentrations that induce anti-proliferative effects. In order to fulfil the

requirements for minimal biological variance our standard operation procedure for test sample generation covers not only the general handling and cultivation of the cells but also the subsequent protein extraction process. Cultivation of HepG2 cells with and without administration of standard compound as control experiment has been carried out five times each. The high number of repeats is necessary to eliminate single and uncontrolled events and ensures that only significant and statistically valid effects on the cell proteome are considered. In order to enable a more precise determination of effective concentrations we started to analyze the standard compounds by additional assays for cell proliferation and cytotoxicity. These assays are based on the quantification of ATP or NADPH dehydrogenases which are indicators for metabolically active cells. Although, these assay systems are quite expensive we will apply them as a supplemental approach towards the characterization of cytotoxic properties of natural products deriving from our in-house screening activities.

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„Extreme und ungewöhnliche Habitate als Quellen neuer pharmazeutischer Wirkstoffe mikrobiellen Ursprungs" Teilprojekt: „Suche nach Wirkstoffen für die Tumortherapie" (0312848A)
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Bundesministerium für Bildung und Forschung
„Hochdurchsatz-Monitoring zur Funktionalen Analyse von Naturstoffen: Neue biologische aktive Substanzen aus chinesischen Heilpflanzen und Schwämmen und ihren assoziierten Mikroorganismen" (0312849A)
Susanne Grabley

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„Marine Pilze II: Charakterisierung und Evaluierung von neuen Wirkstoffen für die Krebstherapie und die Behandlung von Therapieresistenten bakteriellen Infektionen; Kultivierung und Wirkstoff-Identifizierung" (03F0401A)
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Naturstoffpool II
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Industry
CyBio AG
Lagerung sowie Experimente und Studien zur Anzucht und Kultivierung der AequorinScreen Cell Line Expressing the Human Recombinant Orexin 2 Receptor (hOX2-A2)
Isolierung und Strukturaufklärung von Naturstoffen
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(HKI authors in bold)

Han L, **Huang X**, **Sattler I**, **Möllmann U**, Fu H, Lin W, **Grabley S** (2005) New Aromatic Compounds from the Marine Mangrove *Bruguiera gymnorrhiza*. *Planta Medica* 71, 160-164.

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Research Group Drug Testing

Research Group Drug Testing

The Research Group Drug Testing is engaged scientifically both in pharmacological and toxicological investigations of natural substances and synthetic derivatives isolated in the HKI and in the biology of infection of pathogenic (L2/S2) microorganisms. The research group has a broad spectrum of pharmacological, toxicological and experimental evaluation of infection diseases *in vitro* and *in vivo* test models, which are used in the context of the HKI "Internal Product Line" (IPL) and in special research projects. The use of embryonated hen's eggs as alternative to animal experiments reduces significantly the number of laboratory animals used in the fields of pharmacology, toxicology and infectiology.

The main task of the Research Group Drug Testing is the pharmacological and toxicological examination of natural products isolated at the HKI and their synthetic derivatives as well as the preliminary efficacy testing of new lead compounds. Assays for virulence as well as antimicrobial, antiviral, and antifungal activity have been established and are routinely used. In addition it offers its know-how to external partners.
The Research Group Drug Testing is highly engaged in the HKI Internal Product Line (IPL) by performing tests of novel natural products from microbial and plant sources and contributing to the biological profiling of these new products and their synthetic derivatives.

INTRODUCTION | EINLEITUNG

Head:
Dr. Albert Härtl

Die Arbeitsgruppe Wirkstoffprüfung untersucht in verschiedenen Forschungsprojekten die pharmakologischen und toxikologischen Wirkungen von am HKI isolierten Naturstoffen und den daraus gewonnenen synthetischen Derivaten. Weiterhin führen wir infektionsbiologische Untersuchungen mit Mikroorganismen der Risikoklassen L2/S2 durch. Wir verfügen über ein breites Spektrum pharmakologischer, toxikologischer und infektiologischer Testmodelle, die uns die umfassende vorklinische Charakterisierung von neuen Wirkstoffen gestatten. Im Rahmen der „Durchgehenden Bearbeitungslinie" (DBL) und diverser anderer Forschungsprojekte bereiten wir die kommerzielle Verwertung neuer Leitstrukturen vor.

Ein Schwerpunkt unserer Forschungsaktivitäten liegt auf den Pathogenitätsmechanismen humanpathogener Pilze und der Evaluierung neuer antifungal wirksamer Substanzen.

In dem Bestreben, praktikable Alternativen zu herkömmlichen Tierexperimenten zu entwickeln, werden embryonierten Hühnereier eingesetzt. Damit läßt sich die Zahl der eingesetzten Versuchstiere signifikant verringern.

Scientific Projects

1 Pharmacological and Toxicological Evaluations: Biological Profiling of New Natural Products and Synthetic Derivatives and Safety Testing

In this aspect the research group provides assays for examination of antioxidative properties such as xanthine oxidase and horseradish peroxidase and assays for measurement of inhibition of the inflammatory enzymes e.g. 3α-hydroxysteroid dehydrogenase, cyclooxygenase 1 and cyclooxygenase 2 in cell-free systems.

Further possibilities for a pharmacological evaluation of natural products and synthetic derivatives exist by testing their influence on cardiac function and the blood circulation in rats and rabbits, by testing their pro- and antiphlogistic activities in mice or rats, by testing their irritative, angiogenic or antiangiogenic activities on the CAM of chick embryo and also by evaluating their pharmacokinetic properties in mice, rats and rabbits.

Toxic effects of identified lead compounds and products were analysed in special programs, e.g. in a test system with erythrocytes (haemolysis). In special cases the acute toxicity was determined in embryonated hen's eggs, mice and rats.

2 Antimicrobial Activities

For the early stages of antiinfective drug development two different *in vivo* models are currently available. First, an alternative *in vivo* model of 10-day-old chicken embryo is used as a short-term basic screening test to determine the antiviral, antibacterial or antifungal activity of natural products and synthetic derivatives. In addition, this model allows also the determination of the relative virulence among laboratory and epidemic strains of yeasts, fungi, bacteria or viruses. The embryonated hen's eggs testing systems reduces the number of small laboratory rodents in infection experiments. Normally Gram-positive bacteria (e.g. *Staphylococcus* sp., *Streptococcus* sp.), Gram-negative bacteria (e.g. *Pseudomonas* sp., *Klebsiella* sp.) and different pathogenic yeasts or fungi (e.g. *Candida* sp., *Aspergillus* sp.) are used in this alternative infection model. In the same way the virulence of clinically relevant RNA- and DNA viruses (*influenza* virus *A*, *vaccinia* virus and *herpes simplex* virus type 1) was determined. Second, compounds with proved antiinfective activity as determined in the basic screening test are evaluated in mouse infection models. In these models immunocompetent or immunocompromised mice are used. For instance, after reintegration of a mutated gene of *Candida* strain CaVPS34 into the genom of *Candida albicans*, the reconstitution of virulence have been tested. To evaluate the virulence of wild strains in comparison to different mutants different mouse models are used.

In 2004 and 2005 the location of the research group, laboratory house 3, was rebuilt in an ultra-modern building for infection research.

For mouse infection models a new housing system with individually ventilated cages (IVC) was purchased in 2005. This housing system permits the simultaneous working with different pathogenic microorganisms in one experimental animal room.

Conclusions

Determinations of biological activities of novel natural products from microbial and plant sources are performed in various assay systems which provide promising means for a disclosure of new biological properties of natural products and synthetic derivatives. This field addresses numerous questions concerning the mode of action and the structure-activity relationships in close cooperation with other groups engaged in medical and biological research.

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Selected publications
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Härtl A, Sauerbrei A, Stelzner A, Wutzler P (2004) Influenza Infection of the Embryonated Hen's Egg. *Arzneim Forsch Drug Res* 54, 130-134.

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Junior Research Group
Molecular Antibiotics Biosynthesis

Junior Research Group
Molecular Antibiotics Biosynthesis*

Functional Analysis of Genes and Proteins Involved in Biosynthesis of Antibiotics with Mixed Structures.

Most members of the actinomycetes are characterized by a significant physiological differentiation process that normally takes place in parallel to a morphological differentiation. Among other bioactive compounds, a lot of interesting antibiotics are produced during this secondary metabolism indicating that actinomycetes represent an important resource for isolation of novel bioactive compounds with potential for drug development. In recent years, an increasing number of multiresistant pathogens have been observed in medical treatment of bacterial infections. Therefore, the development of new and potent antibiotics is a real necessity. To address this problem, the junior research group for Molecular Antibiotics Biosynthesis focus on the analysis of the biosynthesis of antibiotics with mixed structures such as the peptide/polyketide antibiotics aurantimycin and verucopeptin or the lipopeptide antibiotic friulimicin. In addition to the functional analysis of structural genes, we are also interested in the analysis of the regulation of the biosynthesis of these compounds.

Based on the results of these approaches, it will be possible to carry out targeted modifications in order to generate antibiotics with new structures as well as to improve the quantitative or qualitative production of an antibiotic in the corresponding producer strains.

INTRODUCTION | EINLEITUNG

Head:
Dr. Dirk Schwartz

*until September 2005

Die meisten Vertreter der Aktinomyceten sind durch einen ausgeprägten physiologischen Differenzierungsprozess gekennzeichnet, der in der Regel parallel zu einer morphologischen Differenzierung stattfindet. Während dieses Sekundärmetabolismus werden neben anderen bioaktiven Substanzen auch eine Vielzahl interessanter Antibiotika gebildet. Dadurch stellen Aktinomyceten eine wichtige Quelle zur Isolierung neuer bioaktiver Naturstoffe, aus denen Arzneimittel entwickelt werden können, dar.

In den letzten Jahren wurde bei der Behandlung von bakteriellen Infektionen ein Ansteigen an multiresistenten pathogenen Keimen beobachtet. Daraus ergibt sich die dringende Notwendigkeit zur Entwicklung neuer, besser wirksamer Antibiotika. Die Nachwuchsgruppe Molekulare Antibiotikabiosynthese widmet sich dieser Problematik durch die Analyse der Biosynthese von Antibiotika mit gemischten Strukturen, wie z. B. die Peptid/Polyketid Antibiotika Aurantimycin und Verucopeptin oder das Lipopeptidantibiotikum Friulimicin. Neben der funktionellen Analyse von Strukturgenen interessieren wir uns auch für die Regulation der Biosynthese dieser Substanzen. Um zur Produktion von Antibiotika mit neuen Strukturen oder zu einer qualitativen bzw. quantitativen Verbesserung von Antibiotikabiosynthesen in den jeweiligen Produktionsstämmen zu gelangen, können aufbauend auf den Ergebnissen der durchgeführten Ansätze gezielte Modifikationen vorgenommen werden.

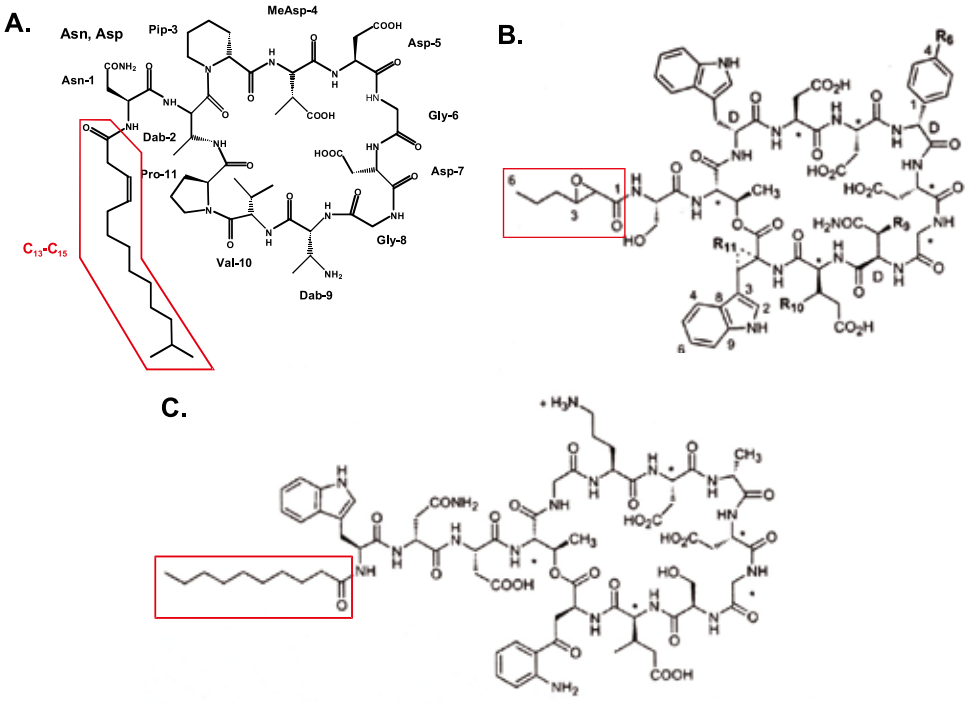

Figure 1
Chemical structures of lipopeptide antibiotics from streptomycetes. A. friulimicin B from *Actinolanes friuliensis*; B. CDA from *Streptomyces coelicolor*; C. daptomycin from *Streptomyces roseosporus*. The positions of the fatty acid residues are marked. MeAsp, methylaspartic acid; Dab, 2,3 diamino butyric acid; Pip, pipecolinic acid.

Our group aims at the molecular genetic and biochemical analysis of antibiotics with mixed structures that are characterized by a combination of a hydrophilic peptide core and a lipophilic side chain (polyketides, fatty acids). Because the biological activities of these antibiotics are affected by the characteristics of the lipid/polyketide part, we are especially interested in the identification of mechanisms involved in the synthesis and linkage of the side chains. Furthermore, the characterization of the regulation of antibiotic biosynthesis as well as the analysis of providing unusual building blocks (e.g. aproteinogenic amino acids) represent another focus of our research.

1 Biosynthesis of the Lipopeptide Antibiotic Friulimicin from *Actinoplanes friuliensis*.

In a screen for new antibiotics against multiresistant Gram-positive bacteria a group of eight lipopeptides was isolated from the rare actinomycete *Actinoplanes friuliensis*. The target of these antibiotics is the bacterial cell wall synthesis, which is probably inhibited by an antibiotic-mediated complexation of the carrier bactoprenylphosphate. The structure elucidation of the antibiotic revealed that all lipopeptides possess an identical macrocyclic peptide as the central element. It is N-terminally linked *via* 2, 3 diamino butyric acid (Dab) either to an acylated asparagine or aspartic acid residue. (Figure 1A, Vertesy at al., 2000)

Despite the differences in both the lipophilic side chains and the exocyclic amino acid residue(s), the isolated compounds show significant similarities to other lipopeptide anti-

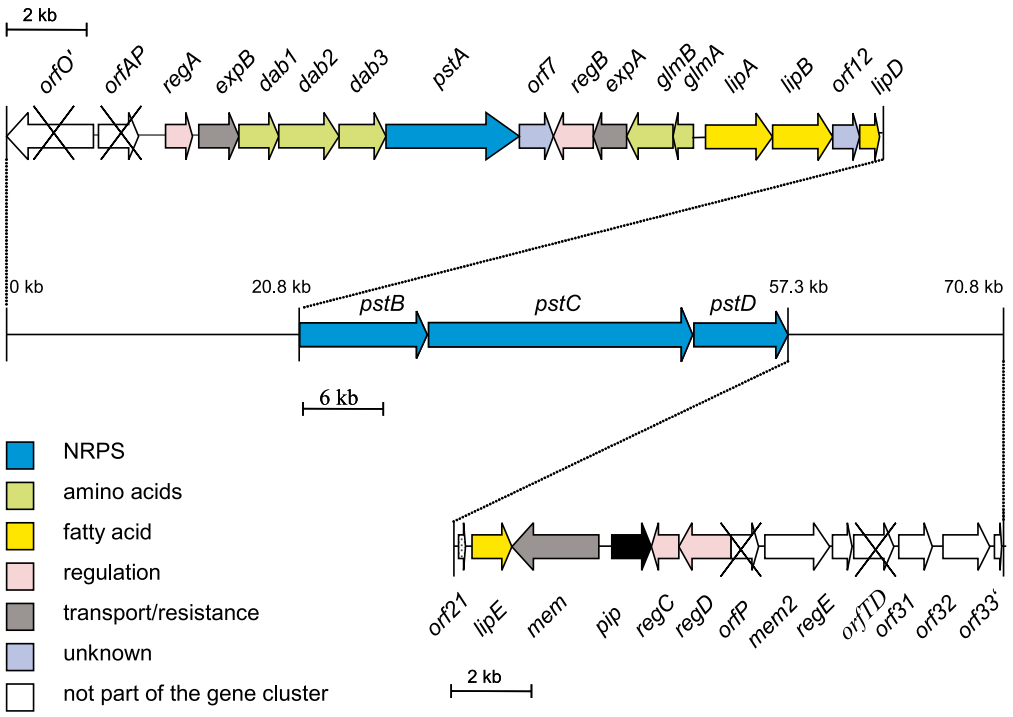

Figure 2
Organization of the friulimicin biosynthetic gene cluster in *Actinoplanes friuliensis*. NRPS, genes encoding non-ribosomal peptide synthetases; amino acids, genes probably involved in the synthesis of non-proteinogenic amino acids; fatty acid, genes proposed to be involved in the synthesis and linkage of the fatty acid component; regulation, genes encoding regulatory proteins; transport/resistance, genes probably involved in transport and resistance; unknown, genes whose deduced products show weak or no similarity to proteins of known function. Genes blocked out with an X were knocked out without interfering with friulimicin biosynthesis.

biotics from streptomycetes such as CDA from *S. coelicolor* (Figure 1B) or daptomycin from *Streptomyces roseosporus*. (Figure 1C).

Whereas four lipopeptide structures (A1437A, A1437B, A1437E, A1437G) are identical with that of the known peptide antibiotics such as amphomycin, tsushimycin, and aspartomycin respectively, the other four lipopeptides represent a new class of antibiotics called friulimicin A-D (Vertesy et al., 2000).

In addition to proteinogenic amino acids, the peptide core of friulimicin is characterized by the existence of the unusual amino acids DAB, pipecolinic acid and methylaspartic acid (L-threo-βmethylaspartic acid).

The complete biosynthetic gene cluster of 24 open reading frames was sequenced and analysed. The boundaries were determined by gene inactivation experiments. (Figure 2)

The cluster contains genes for non ribosomal peptide synthesis, synthesis and linkage of the fatty acid part, transport, resistance and the synthesis of the non proteinogenic amino acids pipecolinic acid (Pip), methyl aspartic acid (Met-Asp) and 2,3-diaminobutyric acid (2,3 Dab). The gene *pip* encodes a lysin cyclodeaminase and was analysed by inactivation and heterologous expression. Stereoselective production of Pip acid in the heterologous host was found. Inactivation of *dab1* and *dab2* demonstrate their involvement in the synthesis of 2,3-Dab. First hypotheses for synthesis mechanisms are postulated.

Furthermore, four regulatory genes (*regA*, *regB*, *regC/D*) were identified within the biosynthetic gene cluster. Their involvement in the friulimicin biosynthesis was shown by gene inactivation resulting in non-antibiotic producing mutants. Whereas RegA and RegB represent pathway specific regulators, the two component regulation system RegC/D affects both physiological and morphological differentiation in *A. friuliensis*.

2 Identification and Characterization of Biosynthetic Genes Involved in the Synthesis of the Antibiotics Aurantimycin and Verucopeptin.

In 1994 the antibiotic aurantimycin was isolated from *Streptomyces aurantiacus* JA 4570 by Gräfe *et al.* (1995). It is related to known hexadepsipeptide antibiotics such as azinothricin, variapeptin, citropeptin and verucopeptin. These compounds display antibacterial and cytotoxic as well as inhibitory effects on cellular adhesion (Sugawra et al., 1993). Like all members of this group of antibiotics, both aurantimycin and verucopeptin are characterized by the presence of piperazinic (PIP)/dehydropiperazinic acid, β-hydroxy leucine and a similar hydrophobic side chain as specific common features.

The structure elucidation revealed that the antibiotics are derived from a mixed biosynthetic pathway. The peptide parts are synthesized non-ribosomally by peptide synthetases (NRPS). In contrast, type I polyketide synthases (PKSI) seem to be involved in the synthesis of the unusual polyketide side chains.

By a reverse genetic approach using NRPS and PKSI specific probes, the aurantimycin biosynthetic gene cluster was identified carrying both polyketide synthase and peptide synthetase genes. So far, an approx. 35 kb DNA fragment of the cluster has been characterized by sequence analysis. On this fragment, 15 ORFs have been found. In addition to NRPS genes encoding for six amino acid activating domains and to PKSI genes other biosynthetic genes were identified which are probably involved in conferring of self resistance (ABC transporter genes) or in the formation of hydroxylated biosynthetic intermediates (hydroxylase gene, P-450 monooxygenase gene).

Using different aurantimycin biosynthetic genes as a probe, the verucopeptin biosynthetic gene cluster was isolated and so far partially characterized.

By comparison of gene clusters of the two antibiotics, we will be able to identify mechanisms that are involved in the formation of the common structural features of these compounds.

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Bundesministerium für Bildung und Forschung
Second phase of the Genomik-Network (BMBF)
(since June 2004): „Analyse der Biosynthese gemischter Sekundärmetabolite in Streptomyceten" within the cluster „Sekundärmetabolit-Biosynthesegene aus Streptomyceten: Aufklärung der Funktion und Regulation sowie ihre Verwendung zur Synthese modifizierter Polyketide" (031U213D)
Dirk Schwartz / Christian Hertweck

Selected publications
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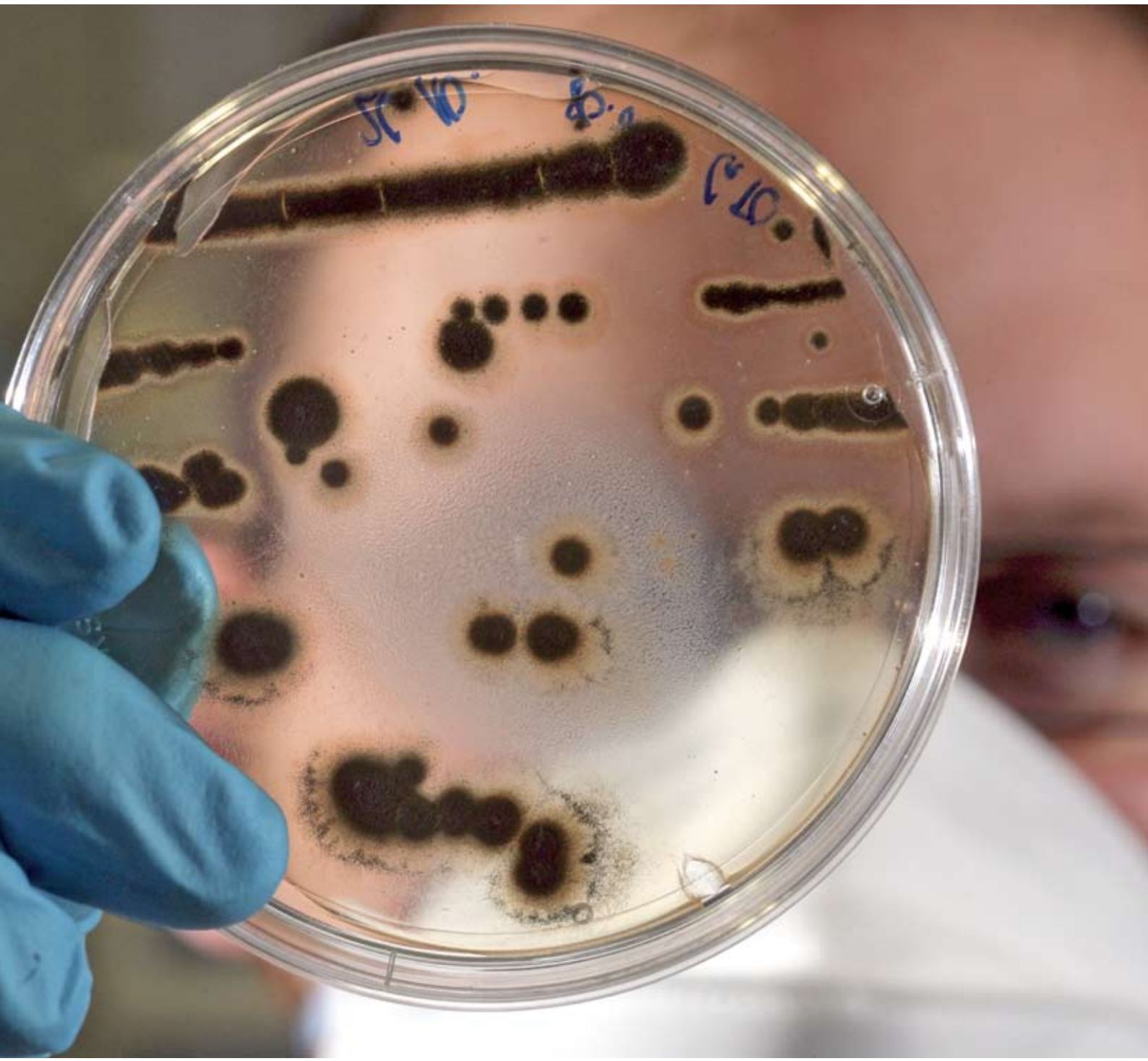

Junior Research Group
Growth-Control of Fungal Pathogens

Junior Research Group
Growth-Control of Fungal Pathogens

Analysis of the Molecular Mechanisms that Regulate Polarized Cell Growth in Filamentous Pathogenic Fungi *Candida albicans* and *Ashbya gossypii*.

Candida albicans is one of the most prevalent human fungal pathogens that occurs as a relatively harmless commensal e.g. in the gastro-intestinal tract of our body, but which can cause severe infections in immuno-compromised patients. *C. albicans* is a dimorphic fungal pathogen which can change its growth form from unicellular yeast-like growth to true hyphal stages. This morphogenetic switching is a key aspect of the virulence of this fungus. Since treatment of *C. albicans* infections relies on only few chemical compounds we aim at understanding the molecular mechanisms that lead to hyphal differentiation and septation in order to identify pathways that could be useful for antifungal drug therapy.

Using the related filamentous fungal model system *Ashbya gossypii* we take a comparative biological approach and try to elucidate the molecular differences of conserved protein networks in different fungi. Functional analyses of fungal target genes are carried out by generating deletion mutants *via* PCR-based methods. Using fusion proteins with the green fluorescent protein (GFP) we analyze the sub-cellular protein localization *in vivo*. High resolution time lapse microscopy is used to monitor endocytic processes and organelle movements.

INTRODUCTION | EINLEITUNG

Head:
PD Dr. Jürgen Wendland

Candida albicans ist einer der wichtigsten humanpathogenen Pilze, der als meist harmloser Kommensale im Magen-Darm Trakt des Menschen vorkommt. In immun-supprimierten Patienten kann *C. albicans* allerdings schwere Infektionen hervorrufen. *C. albicans* ist ein dimorpher Pilz, der sowohl in hefeartigen Stadien als auch als Hyphenpilz vorkommt. Der Wechsel zwischen beiden Wuchsformen ist ein wesentlicher Virulenzfaktor bei *C. albicans*. Für eine Behandlung von *C. albicans* Infektionen stehen derzeit nur wenige chemische Substanzen zur Verfügung. Daher wollen wir molekulare Mechanismen aufklären, die zur Hyphendifferenzierung und zur Septierung beitragen, um neue Signalwege zu identifizieren, die möglicherweise neue Wege für antifungale Therapien eröffnen.

Mit einem verwandten Modellsystem des filamentösen Ascomyceten *Ashbya gossypii* führen wir vergleichend biologische Studien durch, um auf molekularer Ebene Unterschiede in konservierten Protein-Netzwerken in verschiedenen Pilzsystemen aufzudecken. Funktionale Genanalysen führen wir mittels PCR-basierender Disruptionsmethoden durch. Fusionen mit dem Grün-fluoreszierenden Protein (GFP) dienen zur subzellulären Lokalisierung von Proteinen *in vivo*. Hochauflösende Zeitraffer-Mikroskopie erlaubt uns dabei die Darstellung endozytotischer Prozesse und von Organellbewegungen.

1 1G-Protein Signalling and the Actin Cytoskeleton Govern Polarized Hyphal Growth in *Ashbya gossypii* and *Candida albicans*.

Previous studies have shown that GTP-binding proteins of the Ras- and Rho-subfamilies are involved in regulating polarized cell morphogenesis by regulating the dynamic properties of the actin cytoskeleton. Rho-proteins are part of Rho-modules and are activated by binding to GTP. In such an activated state downstream effector proteins are recruited that modulate the actin polymerizing machinery. In our recent studies we focussed on the analysis of proteins that are positioned upstream or downstream of Rho-proteins in signalling to the actin cytoskeleton.

A. gossypii is a filamentous ascomycete which shows sustained polarized hyphal tip growth and frequent initiation of novel growth sites at branches along the extending hyphae. We analyzed the roles of two novel G-proteins, Bud1 and RhoH in *A. gossypii* (Bauer et al., 2004; Walther and Wendland, 2005). Characterization of the *A. gossypii BUD1/RSR1* homolog of *Saccharomyces cerevisiae* revealed that Bud1 localizes to the tip region and is required for the correct localization of the actin cytoskeleton. In the absence of Bud1 hyphal growth was severely retarded and resulted in frequent phases in which growth at the tip stopped. During these phases a polarisome marker, Spa2, was found to disassociate with the hyphal tip in bud1 while Spa2 is continuously localized at the tip in growing wild type hyphae. Fluorescent time lapse microscopy showed that growth in *bud1* is only resumed when Spa2 relocalizes to the hyphal tip. Due to the distorted morphology of *bud1* hyphae Bud1 was identified as a key regulator of hyphal growth guidance. In *S. cerevisiae* Bud1 is known to be part of the bud-site selection machinery that determines the placement of new sites of bud emergence.

A. gossypii RHOH was identified due to its close linkage to *AgRHO1* and constitutes a *RHO1* paralog (generated by gene duplication) that is not present in *S. cerevisiae*. Deletion of *RHOH* resulted in increase of tip cell lysis and increased sensitivity against cell wall disturbing agents, e.g. calcofluor. Thus RhoH and Rho1 share overlapping functions in the maintenance of cell wall integrity.

Rho-proteins in their GTP-bound state can interact and activate downstream effector proteins. One key regulator of hyphal growth is the Spitzenkörper or polarisome. As mentioned above, Spa2 is one component of the polarisome. In *S. cerevisiae* other components are the formin Bni1, as well as Bud6 and Pea2. Since hyphal growth is a key virulence factor in *C. albicans* we performed the functional analysis of the *C. albicans BNI1* gene (Martin et al., 2004). Deletion of *C. albicans* BNI1 results in cell polarity defects, bud-site selection and cytokinesis defects during yeast growth and severely crippled hyphal growth, whereas deletion of a second formin, encoded by *CaBNR1* has no discernible defect in hyphal growth. Bni1-GFP was found to persistently localize to hyphal tips in *C. albicans*. Interestingly, *C. albicans* hyphae evidence of a Spitzenkörper was obtained whereas *C. albicans* yeast cells do not show such an organelle. The actin cytoskeleton was found to be essential to promote hyphal growth since in the *bni1* mutant even the introduction of a constitutively active ras^{g13V} allele did not suppress the hyphal growth defects.

2 Organelle Dynamics Studied by *in vivo* Time Lapse Fluorescence Microscopy

In our studies of a second family of Rho-effectors we characterized mutants in the *WAL1* genes of *A. gossypii* and *C. albicans* encoding homologs of the human Wiskott-Aldrich Syndrome Protein (Walther and Wendland, 2004a,b). As was shown in *S. cerevisiae* deletion of WASP results

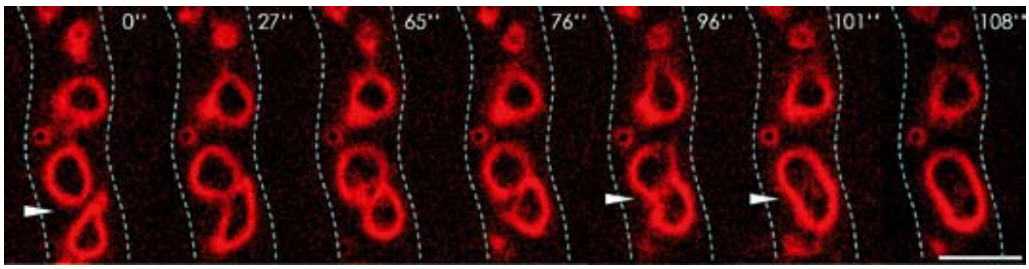

Figure 1
In vivo time lapse microscopy of vacuolar fusion in *Ashbya gossypii*. Hyphal cells were labelled with the vital fluorescent dye FM4-64. A hyphal segment is shown in which one vacuole approaches another. The fusion reaction is completed less than 108 seconds later.

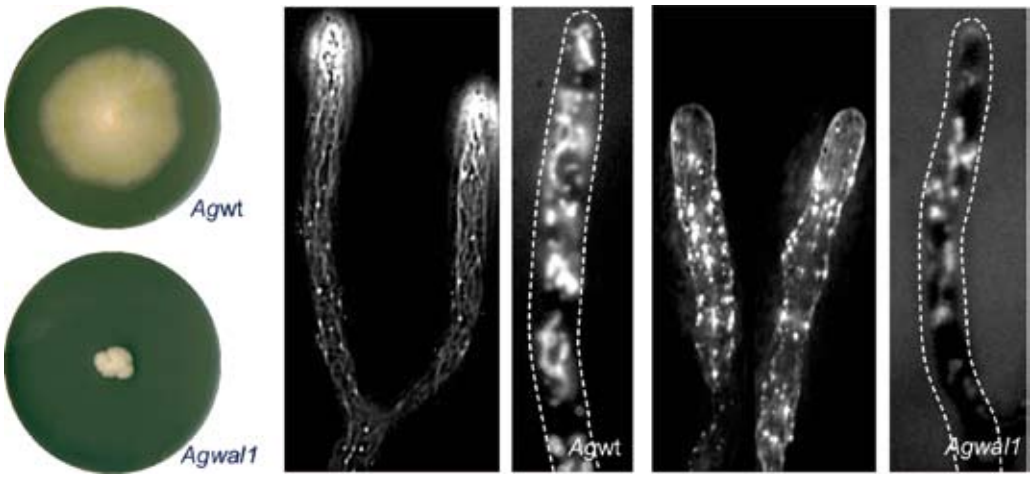

Figure 2
In the *Ashbya gossypii Agwal1* mutant strain cortical actin patches are not localized to the hyphal tip but accumulate in subapical regions. This positioning of patches directs endocytosis away from the hyphal tip to these subapical regions. Deletion of *WAL1* results in defects in endocytosis, vacuole motility and vacuolar movement.

in a defect in endocytosis and in vacuolar morphology. We could show that deletion of WASP abolished hyphal growth in *C. albicans* and severely reduced the polarized growth rate in *A. gossypii*. High resolution fluorescence time lapse microscopy showed that the organelle dynamics of *A. gossypii* vacuoles and the motility of vacuoles were drastically slowed down in *A. gossypii* wal1 hyphae. Similar results were obtained with the *A. gossypii* p21-activated kinase mutant *cla4* indicating that Cla4 and Wal1 constitute a pathway that coordinates hyphal tip growth and endocytosis in filamentous fungi. The role of Wal1 in organelle movement was found to be specific for vacuoles since positioning and movement of mitochondria was not affected in *wal1* hyphae.

As was shown for *C. albicans bni1* mutants, *C. albicans wal1* mutants were also defective in hyphal morphogenesis and this defect could not be suppressed by a constitutive ras allele. Furthermore, in a mouse model of systemic infection *wal1* cells were found to be reduced in virulence.

In summary, our results of the characterization of WASP-homologs showed that filamentous growth requires not only directed delivery of secretory vesicles to the hyphal tip but also coordinated endocytosis. The protein network involved in the regulation of both transport processes requires the polarisome and WASP and may be dependent on the activation of the central cell polarity establishment Rho-protein Cdc42.

These results demonstrated the usefulness of long term fluorescence time lapse microscopy in filamentous fungi. We undertake continuous efforts to improve the tools available to be able to monitor protein localization and organelle movement and are among the leading groups in the fungal field.

As part of these efforts we recently analyzed nuclear distribution and movement in the *C. albicans* wild type and in a *C. albicans* mutant that is defective in correct nuclear distribution due to the deletion of the dynein heavy chain encoding gene *DYN1* (Martin et al., 2004).

3 Functional Analysis of Chitinase Encoding Genes in *C. albicans*

Chitin synthesis and chitin degradation are important processes that are required to maintain cell shape and allow morphogenesis of fungal cells. Chitin synthesis inhibitors are among the few antifungal compounds employed to treat fungal infections. In *S. cerevisiae* the Cts1 chitinase is required to degrade the chitin rich septum to allow separation of mother and daughter cells. Deletion of *CTS1* consequently results in the generation of long chains of unseparated yeast cells. *Cts1* mutants are viable and generate buds which normally indicates that chitinase activity is not required to weaken the cell wall in preparation of bud emergence. *A. gossypii* was found to lack a homolog of *CTS1*. This is in line with its filamentous growth which does not require separation of cells within the hyphal tube. On the other hand, *C. albicans* contains at least three genes, *CHT1*, *CHT2*, and *CHT3* that are homologous to *ScCTS1*. We therefore determined the deletion phenotypes of these genes in *C. albicans* and determined *via* heterologous complementation experiments in *S. cerevisiae* that the *C. albicans CHT3* gene encodes the only functional homolog of *ScCTS1* since only expression of *CHT3* in *S. cerevisiae* was fully able to revert the *cts1* cell separation defect (Dünkler et al., 2005).
These analyses initiated a new line of research in which by functional comparisons of different ascomycetous genomes rewiring of protein networks can be analyzed. This allows elucidating how evolution altered the species specific context of protein networks and will prompt us to investigate differences of yeast and filamentous growth using proteomics tools.

4 Functional Analysis of *C. albicans* Genes by PCR-based Methods

PCR-based gene alteration methods emerged as the preferred tool to manipulate genes in ascomycetous fungi with highly efficient homologous recombination machinery. To be able to carry out different genetic manipulations, e.g. gene deletions, promoter exchanges or the generation of N- or C-terminal fusion proteins using the green fluorescent protein (GFP and its variants) or other tags, an increasing arsenal of vectors has become available. We previously generated a modular set of plasmid vectors for the functional analysis of *C. albicans* genes. In our most recent efforts to also include heterologous marker genes into the *C. albicans* pFA-collection we generated a score of new pFA-plasmids (Schaub et al., 2006).

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„Untersuchungen zu der Rolle von *RHO3* und des WASP-Homologen *WAL1* für die Beibehaltung des polaren Hyphenwachstums bei *Candida albicans*."
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(HKI authors in bold)

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Walther A, **Wendland J** (2004) Polarized hyphal growth in *Candida albicans* requires the WASp homolog Wal1p. *Eukaryot Cell* 3, 471-482.

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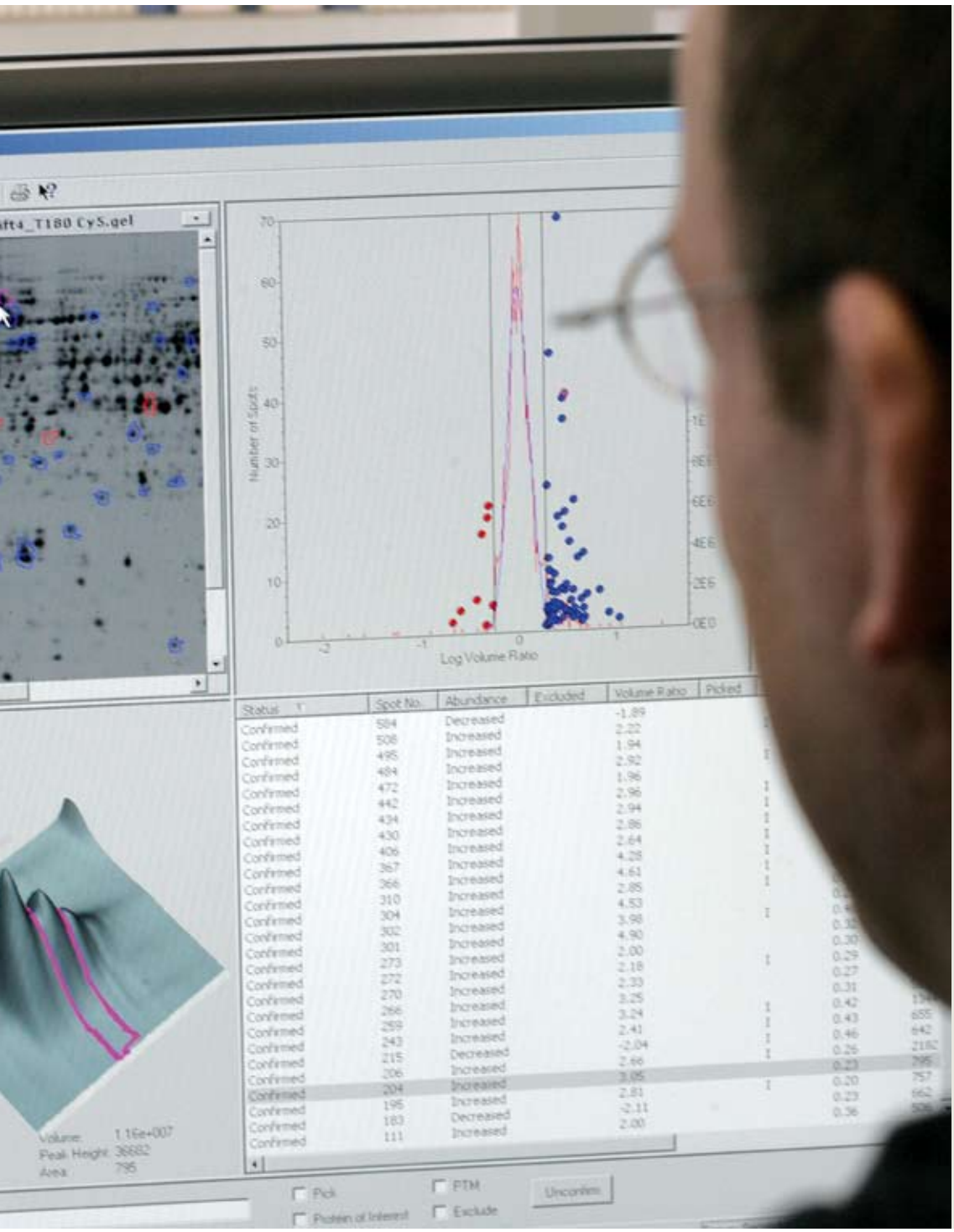

Junior Research Group
Bioinformatics – Pattern Recognition

Junior Research Group
Bioinformatics – Pattern Recognition

Robust Computational Learning of Dynamic or Prognostic Patterns from High-Dimensional Post-Genomic Data

The analysis of molecular biological data generated by high-throughput technologies (in particular, DNA microarrays) requires a number of decisions: the choice of algorithms, the setting of algorithmic parameters, including an adequate search or computation effort, as well as the combined interpretation of usually multiple partial results. Our research group developed solutions in order to relieve the analyst of such decisions or to facilitate decision-making. This was achieved by methods of robust (function-based) optimization, stability analysis of results, comparative analysis of algorithms (benchmarking), and methods for the visualization of complex results. In addition, specific methods were developed and/or configured for problems of biomedical research.

INTRODUCTION | EINLEITUNG

Head:
Dr. Ulrich Möller

Die Analyse molekularbiologischer Daten aus Hochdurchsatzverfahren (insbesondere DNA-Microarrays) erfordert eine Reihe von Entscheidungen: die Auswahl von Algorithmen, die Einstellung von Algorithmus-Parametern, einschließlich eines angemessenen Such- bzw. Rechenaufwands, sowie die gemeinsame Interpretation oftmals vieler Teilergebnisse. Unsere Arbeitsgruppe entwickelte Lösungen, um dem Auswerter solche Entscheidungen abzunehmen oder zu erleichtern. Dies erfolgte durch Methoden der robusten (funktionen-basierten) Optimierung, der Stabilitätsanalyse von Ergebnissen, der vergleichenden Leistungsanalyse von Algorithmen und durch Methoden zur Visualisierung komplexer Ergebnisse. Darüber hinaus wurden problem-spezifische Methoden für Fragestellungen aus der biomedizinischen Forschung entwickelt und konfiguriert.

Scientific Projects

1 How to Find the Most Characteristic Patterns in Data: Solutions from Multi-Objective Strategies

Many post-genomic studies include the hypothesis that the data generated in these studies will exhibit prognostic or dynamic patterns, but number and details of these patterns remain to be discovered a posteriori from the data. We considered the case where a set of characteristic patterns is best described by complementary criteria, but an efficient (clustering) algorithm is available only for optimizing one (master) criterion. Two strategies were developed to cope with the so-called NP hard computation problem. One is based on (evolutionary) optimization of the master criterion. Several parallel evolutionary runs are observed and the process is stopped if the other criteria arrive at unequivocal decisions. The majority decision determines the final solution (Guthke et al. 2005). The second strategy identifies a set of candidate patterns for which complementary criteria likely reach their joint optimum in repeated runs of the evolutionary search process (Möller 2005). Results for simulated and microarray benchmark data (e.g., different tumor types and yeast cell cycle phases) were obtained showing that these approaches can outperform state-of-the-art methods. Both strategies are broadly applicable, where target criteria and evolutionary algorithms can be selected by the user.

2 Where are Consistent Results: Graphical Re-ordering of Labels Assigned to Molecular Objects in Alternative Studies

If life scientists investigate molecular objects, they have to deal with large sets of code labels inevitably required for an efficient electronic bookkeeping. Investigations (e.g. analysis of gene expression) yield labels indicating the assignment of objects to categories (functions, pathways, or co-expression). Each object is often characterized by a set of labels, for example, to consider different molecular features or to ensure robustness of statistical classifiers. We developed visualization tools that give a quick overview of a large assignment table by providing a one-page color image. Co-assignments are indicated by the same color making coherent label subsets easily recognizable. Providing the display required solutions to alignment problems and the choice of a coloring strategy. Approaches from combinatorics, set theory, and optimization theory were used to rearrange the labels for obtaining the clearest view to occurring co-assignments. An advantage of this visualization approach is that all individual assignments of all label generators are simultaneously perceivable at a glance (only limited by graphical resolution). Hence, the experimenter can estimate the more global coherence between the results of different label generators but, nevertheless, keeps reference to each individual object of interest. Both is desirable in molecular biology but hardly achieved by summary statistics. The tools assist in the cumbersome screening of results generated by large-scale molecular investigations and in checking the biological plausibility of their computational results. (Figure 1)

3 Benchmarking: About the Strengths and Weaknesses of 'Workhorse' Algorithms

Post-genomic data analysis makes frequent use of algorithms transferred from other fields where they have been tested on different tasks and/or less complex data. We investigated the performance of such types of algorithms. A very popular variant of hierarchical clustering was compared with function optimization schemes (Radke and Möller 2004). Both approaches (the first implicitly and the second explicitly) aim at

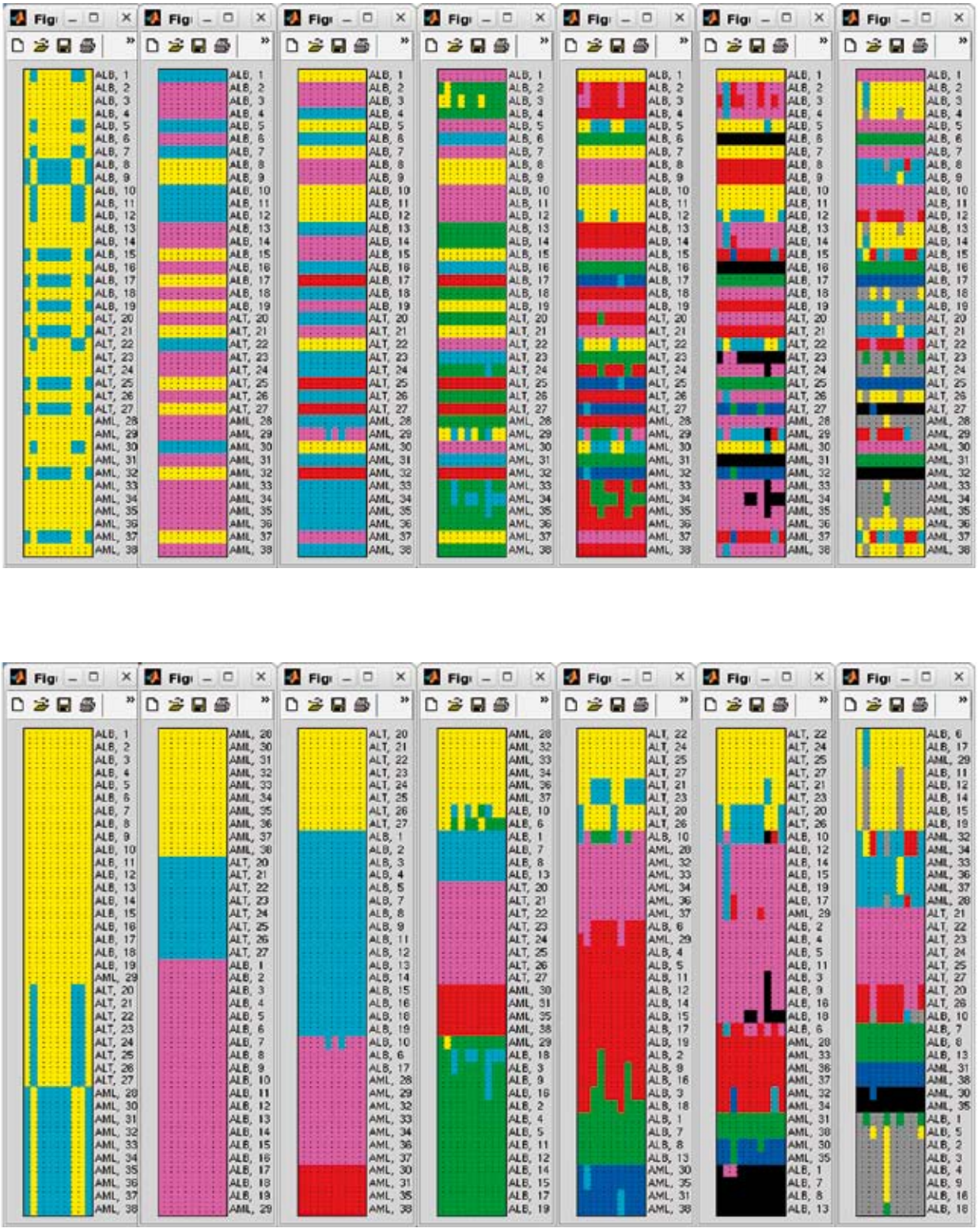

Figure 1

Visualization (color-map) of 70 clustering results (columns) obtained for 38 cases of Leukemia (rows; data of Golub et al., Science, 1999). The goal is to identify possible subclasses of Leukemia in general – not only of the sample under study. 10 data sets were randomly (re)sampled from the original data to assess clustering stability. The clustering algorithm was configured to generate partitions with 2 to 7 clusters for each resample (seven panels in A and B).

A) All results with identical row-ordering of the Leukemia cases that represents a random order of still unclassified patients. B) Results of each panel with a separate row-ordering performed to permit a clear view to stable co-assignments. It is easily recognizable that the partitions in panel two and three from the left were most stable, suggesting three or four robust Leukemia subclasses.

the generation of compact clusters. The optimization schemes were shown to yield consistently more compact clusters (i.e., more characteristic patterns) for simulated and microarray data sets. In another investigation we assessed the performance of data resampling techniques for the discovery of prognostic tumor classes from DNA microarray data. In a recent re-analysis of the largest microarray-based studies on tumor outcome prediction, resampling was shown to be relevant for avoiding over-optimistic results (Michiels et al.; Lancet, 2005, 488-492). Our robust performance ranking provided guidelines how to best resample in upcoming analyses (Möller and Radke, 2006). Moreover, the results inspired the development of novel methods that discover smaller classes more likely and require less computations.

4 What is the Role of Different Cell Types in Disease: Analysis of Gene Expression in Tissues and Isolated Cell Fractions

Biological tissues consist of various cell types that differentially contribute to physiological and pathophysiological processes. Determining and analyzing cell type-specific gene expression under diverse conditions is thus a central aim of biomedical research. Therefore, gene expression profiles in whole tissues were compared to isolated cell fractions purified from these tissues in patients with rheumatoid arthritis and osteoarthritis (cooperation with R. Kinne).

The comparison was done using computationally reconstituted tissue expression profiles that combine the expression profiles of the respective isolated cell fractions according to their relative mRNA proportions in the tissue. The mRNA proportions were determined by trimmed robust regression using only the most robustly expressed genes. The results for the mRNA proportions were also compared to cell proportions determined by immunohistochemistry.

The proposed method of Robust Computational Reconstitution uses an intermediate number of robustly expressed genes to determine the relative mRNA proportions. This avoids the exclusive dependence on the robust expression of individual highly cell type-specific marker genes and, as shown by the present study, the bias towards an equal distribution when using all genes. Once the relative mRNA proportions are estimated gene expression profiles of tissues and isolated cell fractions can be compared to identify robustly expressed genes representing basic metabolism or persistent pathological changes and regulated genes possibly reflecting physiological or pathological cell communication processes. Both are of biomedical interest and can be further screened for pathophysiological relevance.

5 Dynamic Signatures: Gene Expression Patterns for the Modeling of Transcriptional Regulation

Gene regulatory networks are reverse engineered based on experimental expression time courses (e.g. of a stimulus response). Such data sets often suffer from a sparse sampling over time. Then a modeling approach involves constraints concerning the number of parameters that can be reliably fitted. Using our multi-objective clustering strategies, we determined robust sets of gene expression patterns (dynamic signatures) that improved the reconstruction of alternative network models by HKI systems biologists (R. Guthke). This collaboration initiated a novel data-driven modeling pipeline. Currently, the approach is utilized to investigate gene regulation of *Aspergillus fumigatus* (a model organism which is the focus of HKI research).

In a cooperation within the Jena Centre for Bioinformatics (A. Beyer) we determined groups of co-expressed genes in yeast under various experimental conditions, e.g., stress response, cell cycle or sporulation. Members of the identified gene groups which exhibited the same dynamic signature were then used to provide one of several types of evidence that these genes were regulated by common transcription-factor-target interactions.

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Pilot Plant for Natural Products

Pilot Plant for Natural Products

The research of the HKI Pilot Plant for Natural Products is mainly focused on the development, optimization and scale-up of biotechnological processes in particular related to the production of natural compounds from flask cultures *via* laboratory scale to pilot scale.
The team of the HKI 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. Simultaneously, we develop processes for the expression of recombinant proteins based on high-cell-density fermentation.

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. In this respect we cooperate internally with all departments of the HKI, groups of the FSU Jena and the University of Applied Sciences Jena.

INTRODUCTION | EINLEITUNG

Head:
Dr. Uwe Horn

Forschungsschwerpunkte des Naturstoff-Technikums sind die Entwicklung, Optimierung und das Scale-up biotechnologischer Prozesse von Naturstoff-Bildnern von der Petrischale über den Labormaßstab bis zum Pilotmaßstab. Dazu liegen langjährige Erfahrungen auf dem Gebiet der Stammentwicklung, der Kultivierung und Fermentation eines breiten Spektrums prokaryontischer und eukaryontischer Mikroorganismen vor.
Parallel dazu werden Prozesse für die Herstellung von rekombinanten Proteinen entwickelt. Schwerpunkt dabei ist die Hochzelldichte-Fermentation als Fed-Batch-Verfahren für hohe Volumenausbeuten.

Für das Downstream-Processing und die Aufreinigung niedermolekularer Naturstoffe, Biopolymere und Proteine stehen moderne analytische Methoden sowie ein breites Spektrum von Technologien und Equipment zur Verfügung. Um die physiologischen Anforderungen der produzierenden Mikroorganismen zu analysieren und die Fermentationsprozesse zu steuern, werden experimentelle und theoretische Methoden für die Entwicklung von Hochleistungsverfahren kombiniert.
Das Naturstoff-Technikum arbeitet intensiv mit den anderen Forschungsabteilungen des HKI und Gruppen der FSU und Fachhochschule Jena zusammen. Dadurch wird die Bioprozeßentwicklung optimal mit Aspekten der Mikrobiologie, Physiologie und Gentechnologie verknüpft.

Special research fields are:

(i) On-line *in vivo* monitoring of bioprocess parameters *via* genetically engineered reporter systems.

(ii) Screening of new microorganisms using large numbers of microcultures of < 100 nl volume in PTFE capillaries and development of a high throughput bioassay system using microsegmented cell cultures in liquid/liquid-two-phase-systems.

(iii) Selection and expression of recombinant antibodies from a synthetic camelid library, which was optimized for expression in *E. coli*.

In this respect the HKI Pilot Plant offers research cooperation with external partners from academia and industry.

Spezielle Forschungsfelder sind:

(i) On-line *in vivo* Monitoring von Bioprozess-Parametern *via* gentechnologisch hergestellter Reporter-Systeme

(ii) Screening von neuen Naturstoff-Bildnern in Mikrokultursystemen (<100 nl) in PTFE Kapillaren und Entwicklung von High-Throughput Bioassay-Systemen mittels mikrosegmentierter Zellkulturen in flüssig/flüssig-zwei-Phasen-Systemen.

(iii) Selektion und Expression von rekombinanten Antikörpern aus einer vollsynthetischen, optimierten cameliden Antikörperbibliothek.

Das Naturstoff-Technikum bietet optimale Voraussetzungen für F/E-Kooperationen mit Partnern aus anderen akademischen Forschungseinrichtungen und der Industrie.

Scientific Projects

1 Production of Microbial Biomass and Natural Products for the Internal Product Line, Research Projects of HKI Groups and Partners from Academia

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

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.
206 fermentations at the 7 to 300 liter scale were performed within 2004-2005, 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 a synthetic resin column, solvent extraction, and concentration and freeze drying of the extracts. In 2004-2005 products from about 8000 liter fermentation broth were prepared.

Collaborations within the IPL of HKI and with external partners

Project Isolation and characterization of novel rhizoxin derivatives produced by bacterial endosymbionts isolated from *Rhizopus microsporus* strains (HKI, Dept. Biomolecular Chemistry)
Activities at Pilot Plant Pilot scale fermentation of *Burkholderia* strains and downstream processing to produce rhizoxin derivatives for structural elucidation and for studying secondary metabolite profiles of different symbionts

Project Production and characterization of polyketide derivatives by mutasynthesis (HKI, Junior Research Group Bioorganic Synthesis)
Activities at Pilot Plant Pilot scale fermentation of recombinant *Streptomyces* strains and downstream processing to purify aureothin derivatives

Project New bioactive natural products produced by microorganisms isolated from special habitats (HKI, Dept. Molecular Natural Products Research,)
Activities at Pilot Plant Fermentation and downstream processing in 30 to 300 L scale

Project Characterization of *Aspergillus nidulans* mutants using proteome analyses
Activities at Pilot Plant Continuous culture of *A. nidulans* strains to produce mycelia grown at defined reproducible culture conditions

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

Project Potential use of heavy metal resistant streptomycetes in bioremediation (Friedrich Schiller 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.

Project Selection and expression of designed ankyrin repeat proteins (Zürich University Biochemical Institute)

Project Selection and expression of recombinant VHH-antibodies (Leibniz Institute for Age Research - Fritz Lipmann Institute -)

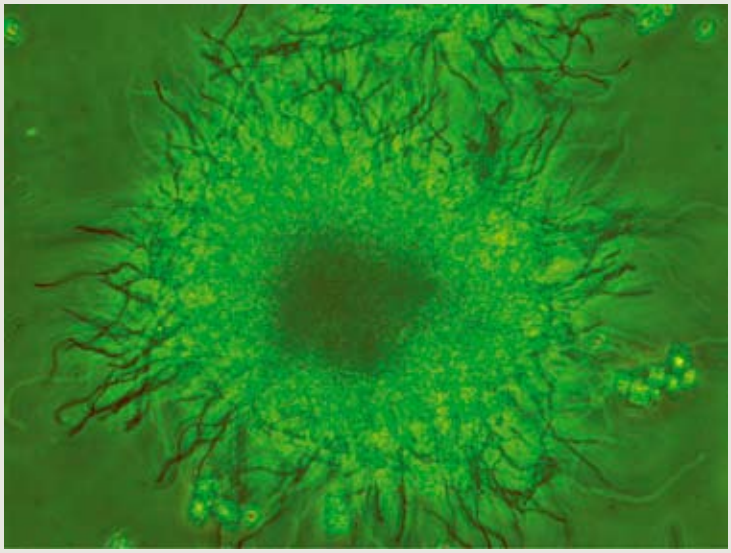


Figure 1
Pellet of *Streptomyces tendae* HKI 0179 producing cervimycins

2 Development of Fermentation and Downstream Processes for Natural Products

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

Fermentation and downstream processes for several natural products (antibiotics, recombinant proteins, biopolymers) were developed within the IPL of HKI and in collaboration with industrial partners.

Cervimycins

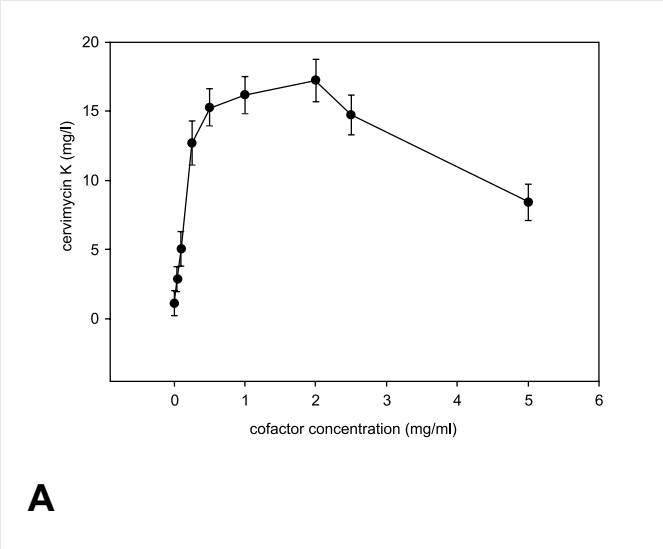
Cervimycins are novel polyketide glycosides produced by a new strain of *Streptomyces tendae* isolated at the HKI. For structural elucidation, toxicological and pharmacological studies, and chemical modifications to be performed in the HKI department Biomolecular Chemistry it has been necessary to produce higher quantities of cervimycins than available by using the basic fermentation process. Therefore process improvement in the 7–75 liter scale has been performed at the Pilot Plant including seed culture and medium optimisation, development of a fed-batch fermentation process and improvement of the HPLC method for quantification of the different cervimycins. The yield of cervimycin K and other cervimycins was increased 5 to 10fold compared with the basic process. The fed-batch process will be scaled up to the 300 liter scale and the downstream processing for the pilot scale production of cervimycins will be improved. (Figure 1)

Antifungal antibiotic

Strain improvement and development of the fermentation and product purification processes for production of an antifungal antibiotic have been performed in collaboration with a Swiss pharmaceutical company.

Diagnostic enzymes

In collaboration with Merck KgaA, Germany, we designed and optimised a large scale fer-



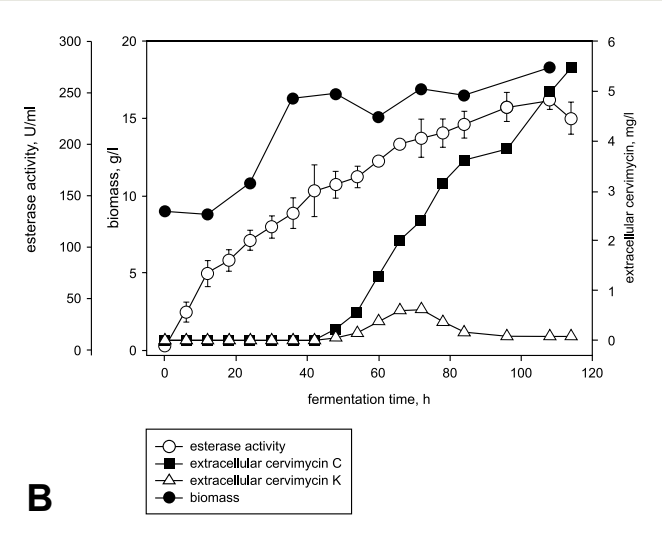


Figure 2
A Dependence of the esterolytic formation of cervimycin K on the concentration of the "cofactor" bovine serum albumin

B Kinetics of unspecific esterase activities in a cervimycin fermentation

mentation and downstream process for diagnostic enzymes.

Biopolymers

In collaboration with Tepha, Inc. (Cambridge, USA) fermentation and purification processes for novel absorbable biopolymers, the polyhydroxyalkanoates poly-4-hydroxybutyrate (P4HB) and the copolymer poly-3-hydroxybutyrate-co-4-hydroxybutyrate (P3HB-co-P4HB), were improved using recombinant *E. coli* K12 strains. The production process for P4HB was scaled up to the 1500 liter scale by the project team of the Pilot Plant.

By filing a Device Master File for P4HB with the FDA, Tepha completed the first steps towards regulatory approval of medical products made from this material. At present efforts at Tepha are focusing on the development and registration of its first medical product, a surgical suture made of TephFLEX™ (P4HB).

3 Production of Cervimycin K by Esterolytic Splitting of Cervimycin C Half-Ester Bonds

Group Leader: Peter-Jürgen Müller

Cervimycin K is a novel polyketide glycoside antibiotic with significant activity against multi-drug-resistant staphylococci and vancomycin-resistant enterococci first described at the HKI. Cervimycin K is produced by *Streptomyces tendae* as a minor component. The major component cervimycin C is a half-ester containing the rare dimethylmalonyl unit attached to the 4' terminus of the tridesoxyhexose tetramer while cervimycin K does not contain this ester bond.

To enhance the yield of cervimycin K it was proposed that esterolytic enzymes process the enzymatic hydrolysis of the half-ester linkage of cervimycin C. This could be a potential biotechnical alternative to the comparable low yields of cervimycin K obtained by

fermentation. (Figure 2)
In this respect we showed that hydrolysis of cervimycin C by the horse liver esterase yielded up to 5 % cervimycin K only. It is known that hydrolysis of dicarboxylic acid diesters particularly of substituted malonylesters by pig liver esterase leads exclusively to the half-esters. Accordingly the second ester bond is relatively stable against hydrolysis because of the high negative charge of the dissociated carboxyl group of the half-ester. Surprisingly it was found that the presence of a special "cofactor"-like, non-enzymatic protein influences the yield of cervimycin K according to a maximum function. The yield reached 20 to 30%. Probably the "cofactor" supports the enzymatic attack by a "substrate activation mechanism". The "cofactor" forms a complex with the deprotonised carboxylate function of the dimethylmalonyl group of the half-ester. In this way the strong negative charge seems to be lowered in the surrounding of the ester bond and is distributed over the whole complex. Such "cofactor" function of a non-enzymatic protein is rare known.
Contrary to expectation the intracellular and extracellular esterase activities formed by *Streptomyces tendae* during the fermentation process are inactive in splitting of cervimycin C also in the presence of the "cofactor".

4 *In vivo* Monitoring of Misfolded Recombinant Proteins in *Escherichia coli* During Fermentation Processes

Group Leader: Uwe Horn

Overexpression of recombinant gene products in the periplasm of *Escherichia coli* frequently results in misfolded proteins often degraded by cellular proteases. Expression and folding of periplasmic recombinant proteins are influenced by a number of parameters like promoter strength, ribosome-binding sites and signal peptides. Folding promoting agents or coexpression of molecular chaperones and folding catalysts have been used to improve protein folding in former studies. Regarding these factors, the successful expression of recombinant proteins in *E. coli* requires technologies for direct folding monitoring during fermentation, which are independent of functional assays.
In contrast to recent studies using protein fusions with GFP or LacZ, our approach uses the native stress response to monitor accumulation of misfolded and aggregated proteins in the periplasm of *E. coli*. Accumulation of misfolded proteins in the periplasm leads to the induction of the serin protease DegP. Based on the *deg*P-promoter and the luciferase reporter gene we developed an on-line technology, allowing *in vivo* kinetic studies of protein misfolding. Moreover, a highly versatile expression vector set for differential transcriptional and translational control of expression was developed and used for solubility screening. We showed that the amount of functional protein is inversely proportional to the on-line luciferase signal. Further, we demonstrated that addition of folding promoting agents and coexpression of a molecular chaperone influence reporter gene activity and thus supports protein folding. Taken together, this technology offers a simple tool for on-line monitoring of recombinant protein folding in fermentation processes.

5 Development and Evaluation of a Novel Microfluidic Technology for Isolating New Microorganisms and High Throughput Drug Screening

Group Leader: Martin Roth

Only few of the microorganisms present in the environment has been cultured yet, e. g. only about 7000 bacterial and archaeal species have been described. It was shown using molecular-biological approaches that millions or billions of microbial species exist in nature. It is assumed that the majority of microbial species could not be cultured due to inappropriate methods of cell separation and culture. Cultivating and characterising microbial cells individually is of particular interest in order to make new and rare species available and to improve screening for new natural products.
In cooperation with partners from Thueringian institutes (project MINIKULT) we have devel-

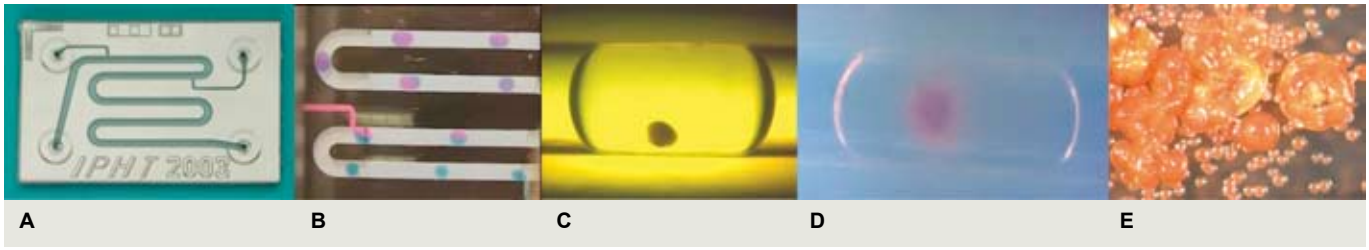


Figure 3
A Microfluidic double injector chip for generation of culture compartments and addition of effector containing solutions

B supplementation of microcultures with growth effectors

C, D colonies of microorganisms grown in culture compartments

E Colonies of a rare actinomycete, *Nonomuraea dietziae*, isolated by the novel technology.

oped a novel technology for parallel cultivation of large numbers of individual microbial cultures in 40–100 nanoliter microcompartments. This includes the following steps:
Generating culture compartments separated by a liquid immiscible with water using a microchannel injector chip device, incubating in PTFE capillaries, detecting growth and finally sorting individual culture compartments automatedly. (Figure 3)

That technology was applied for cultivating microorganisms from soil samples. We are especially interested in the isolation of rare actinomycetes since this group of bacteria is an attractive source of new natural products. Bacteria of diverse phylogenetic origin could grow in those microcompartments described. It was shown that rare species can be isolated using this cultivation approach.
Our results prove that microbiological techniques can be immensely down-scaled to very small culture volumes of aqueous media separated by immiscible liquids. Many of the traditional microbiological methods are potentially adaptable to the described segmented-flow cultivation processes of large sample numbers. Particularly, the search for new microbial species and microbiological assays will benefit from this technology.
Numerous applications within the screening for pharmaceuticals and other active substances are expected. The combination of chemical and cell biological operations in sequences of microcompartments gives very promising possibilities for studying the influence of substances on cells and cell-cell interactions.
In this context the joint project SERIZELL, started in 2005 by eight industrial and academic partners, is directed to development, construction and evaluation of a microfluidic assay system for high throughput screening of biologically active compounds.
It has already been shown that not only microorganisms but also eukaryotic cells proliferate in microcompartments in PTFE capillaries. Consequently eukaryotic cell lines may be applied in drug screening assays to be performed in the new microfluidic system.

6 Selection and Expression of VHH Camel Antibodies from a Full Synthetical Antibody Library

Group Leader: Uwe Horn

The expression of antibody fragments in *Escherichia coli* has become a widely used technique for serveral reasons and it can be advantageously combined with phage-display technology which makes use of *E. coli*. The increasing number of antibody sequences and crystal structures has led to the determination of consensus sequences, which allowing a rapid cloning of recombinant antibody genes and whole antibody libraries.
The molecular format of the antibody fragment plays a key role for the functional expression in *E. coli*. A frequently used format is the so called single-chain-Fv (scFv) fragment, which is characterized by two disulfid bridges one in the VL and one in the VH domain. Obviously, a functional expression requires a translocalization of the whole molecule in the periplasm, which can lead to malfunctions and further optimization steps.
In this respect, we designed a full synthetical VHH camel antibody library, based on an alignment of published sequences of this molecule. The small VHH camel antibodies whith a molecular weight of about 15 kD requires no VL domains for their function and only one disulfid bridge for a stable structure. To obtain a diversity of about $>10^8$ independent clones we designed our library by using 72 sublibraries,

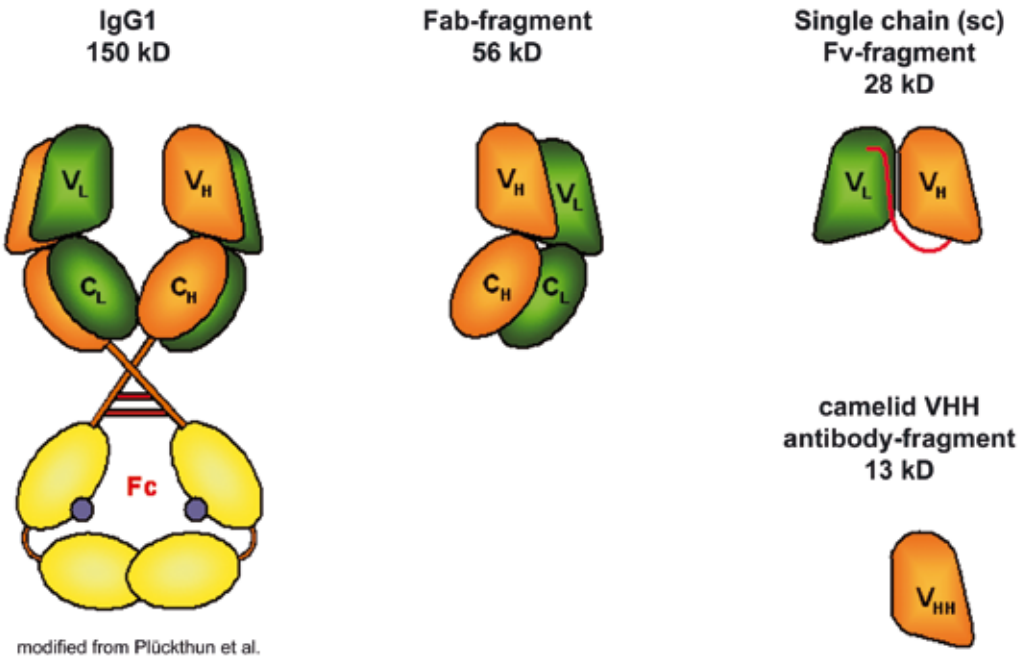


Figure 4
Sizes of the most used biotechnological antibody formats in relation to the camelid VHH antibody-fragment

mainly as a result of the variability of the CDR3 region with lengths of about 5 to 17 amino acids. Furthermore, to achieve a high amount of functional clones, we preselected the whole library as a periplasmic ß-lactamase fusion. Simultaneously, we exclude by this optimization all clones, which contain frameshifts, caused by the assembly PCR, stop codons and leads to misfolded VHH fragments in the periplasm, respectively.

The optimized library was used for the selection of VHH-antibodies against different targets, mainly proteins. In comparison to the immune system, which provides antibodies to any antigen, we are able to select VHH antibodies in a competetive procedure, e.g. to specific protein domains and proteins whith high homology. For this purpose, the non desired antigen was added during the panning (selection) procedure in a five to ten fold surplus to the soluble phase, whereas the target was coupled to paramagnetic beads. Using this technology, we selected currently high specific VHH antibodies to different proteins of the bone morphogenetic protein (BMP) family (in cooperation with Peter Hortschansky, HKI/MAM) and a conformation-dependent antibody to a mature amyloid fibril (in cooperation with the group of Marcus Fändrich, FLI) called B10 (unpublished data). The panning procedure was carried out using immobilised mature Aβ-fibrils and a 5-fold surplus of freshly dissolved β-amyloid(1-40) peptide as competitor. Dot blot analysis confirm that B10AP recognizes non-biotinylated fibrils but does not interact with non-biotinylated, disaggregated peptide. All selected antibodies are characterized by binding properties in a lower nanomolar range. In addition we increased the affinity via fusion of the VHH fragments to the dimeric alkaline phosphatase, which reconstitute the bivalent binding properties of natural antibodies and give us simultaneously an useful enzymatical activity for further applications, e.g. western blots. In cooperation with A. Plückthun, University Zürich, we developed a vector set,

which provides an easy subcloning of our VHH antibodies from the panning vector in different expression vectors, e.g. as VHH single domain and as a fusion with the alkaline phosphatase. By integration of genetically stabilizing elements, the vector set is very suited for the expression in high cell density fermentations HCDF allowing cell densities of about 100 g/L (dry weight) and protein yields in an upper milligram scale. (Figure 4)

7 Inhibitory Activities Against Microbial Hyaluronate Lyase

Group Leader: Peter-Jürgen Müller

Sepsis is a common reason of mortality and morbidity worldwide caused mostly by microorganisms like *Staphylococcus*, *Streptococcus* or *Candida*. The pathophysiology of sepsis is complex and resulting from the effects of circulating bacterial products, mediated by cytokine release, occurring as a result of sustained bacteria. One important pathogenic factor is the enzyme hyaluronate lyase excreted by the causative organism leading to destructing of tissues, spreading of toxic agents, and the unregulated release of chemotactic mediators.

Therefore a new therapeutic origin is the administration of natural specific inhibitors of hyaluronate lyase in the context of classical antiinfecting and inflammatoric strategies. Up to now such natural inhibitors with high specific inhibitory activity are unknown.

The Pilot Plant of Natural Products of HKI can produce beside the lyases from *Streptococcus* agalactiae and *Streptococcus equisimilis* almost unlimited amounts of recombinant hyaluronate lyase of the *Streptococcus agalactiae* – enzyme. The screening of new inhibitors in plant materials was complicated by the presence of tannic substances, acting as unspecific inhibitors. In consequence a specific screening test was developed. In course of a limited screening program some highly active extracts were found in materials from plants and mushrooms. An outstanding inhibitor was found in plant material with an inhibitory activity of IC_{50} = 0.1 µM against hyaluronate lyase from *Streptococcus agalactiae* with a low inhibitory activity against bovine hyaluronidase.

It was shown that remarkable differences exist between the inhibitory activities of inhibitors in dependence on the origin of the hyaluronic acid-splitting enzymes. Indeed the recombinant enzyme that only differ in the structure from the microbial enzyme by a his-tag has a different inhibitory behaviour.

Group members

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External funding

Deutsche Forschungsgemeinschaft
„Mikrobielle Wechselwirkung mit Werkstoffoberflächen» (KN 468/1-3)
Wolfgang Knorre

Deutsche Forschungsgemeinschaft
"Multiple functions of the phosphatidylinositol-3-kinase Vps34p of the human pathogenic yeast *Candida albicans*" (SFB 604/B1)
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Bundesministerium für Bildung und Forschung
„Mikroreaktorik für High Throughput-Einzelzellkulturen von Mikroorganismen – MINIKULT";
Teilvorhaben: „Systemevaluierung" (16SV1373)
Martin Roth

Bundesministerium für Bildung und Forschung
AiF-Projekt „Aufnahme systemtypischer Aromenmuster zur Indentifikation von Prozesszuständen und mikrobiellen Mono- und Mischkulturen zwecks Prozessüberwachung"
(KF 0181107KMD2)
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Bundesministerium für Bildung und Forschung
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Martin Roth

Industry and Bundesministerium für Bildung und Forschung
Lehmann Maschinenbau GmbH
Verbundvorhaben „Technische Erprobung und Überführung einer Laboranlage zur solaren Trinkwasserentkeimung in Entwicklungsländern"
(02WT0054)
Martin Roth

Industry
Biosynergy GmbH
„Bewertung der Reproduzierbarkeit der Biosynthese von ß-Carotin mittels *Blakeslea trispora*"
Arnulf Christner, Peter-Jürgen Müller

Industry
Tepha Inc.
„Improvement and Scaling-up of Fermentations to Produce Polyhydroxyalkanoates"
Martin Roth

Industry
VitraBio GmbH
„Funktionalisierung poröser Glasoberflächen"
Martin Müller

Industry
kreAktiv GmbH
„Herstellung von Pflanzenextrakten und deren Testung auf antiinflammatorische und antioxydative Wirkung"
Waltraud Hertel

Industry
M.E.A. Mauf und Rudow GmbH
„Durchführung von Versuchen zur Reduzierung von Lebendkeimzahlen von *Escherichia coli* in Leitungswasser"
Martin Roth

Industry
Swiss company
„Untersuchung von Bakterienstämmen für die biotechnologische Herstellung eines antifungalen Antibiotikums"
Martin Roth

Industry
Swiss company
„Verfahrensentwicklung und Verfahrensoptimierung für die biotechnologische eines antifungalen Antibiotikums"
Martin Roth

Industry
Merck KgaA
"*E.coli* Expression of diagnostic enzymes"
Uwe Horn

Industry
MoBiTec GmbH
„Evaluierung einer vollsynthetischen *Cameli*-dae-VHH-Antikörperbibliothek aus *Escherichia coli* für die Selekrion von rekombinanten VHH-Antikörpern gegen spezifische Fc-Fragmente natürlicher IgG"
Uwe Horn

Industry
Carl-Zeiss Meditec AG
„Untersuchungen zur Wirksamkeit von antifungalen Ausrüstungen von Optikbauteilen der Carl-Zeiss Meditec AG"
Martin Roth

Industry
optiferm kempten GmbH
„Fermentation von Propionibakterien"
Arnulf Christner

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(HKI authors in bold)

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Schön T, Grodrian A, Metze J, Henkel T, Köhler JM, **Martin K**, **Roth M** (2004) Mikroreaktorik für die High Throughput-Einzelzellkultivierung von Mikroorganismen. *Bioperspectives* 8, 77.

Collaborations

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Jenoptik Mikrotechnik GmbH, Jena
F. Reuter

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Merck KgaA, Darmstadt

Tepha, Inc. Cambridge, USA

Till Photonics GmbH, München
Prof. Dr. R. Uhl

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 drugs.

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 pharmakologisch 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 Suche nach neuen Leitstrukturen. Für Natur-

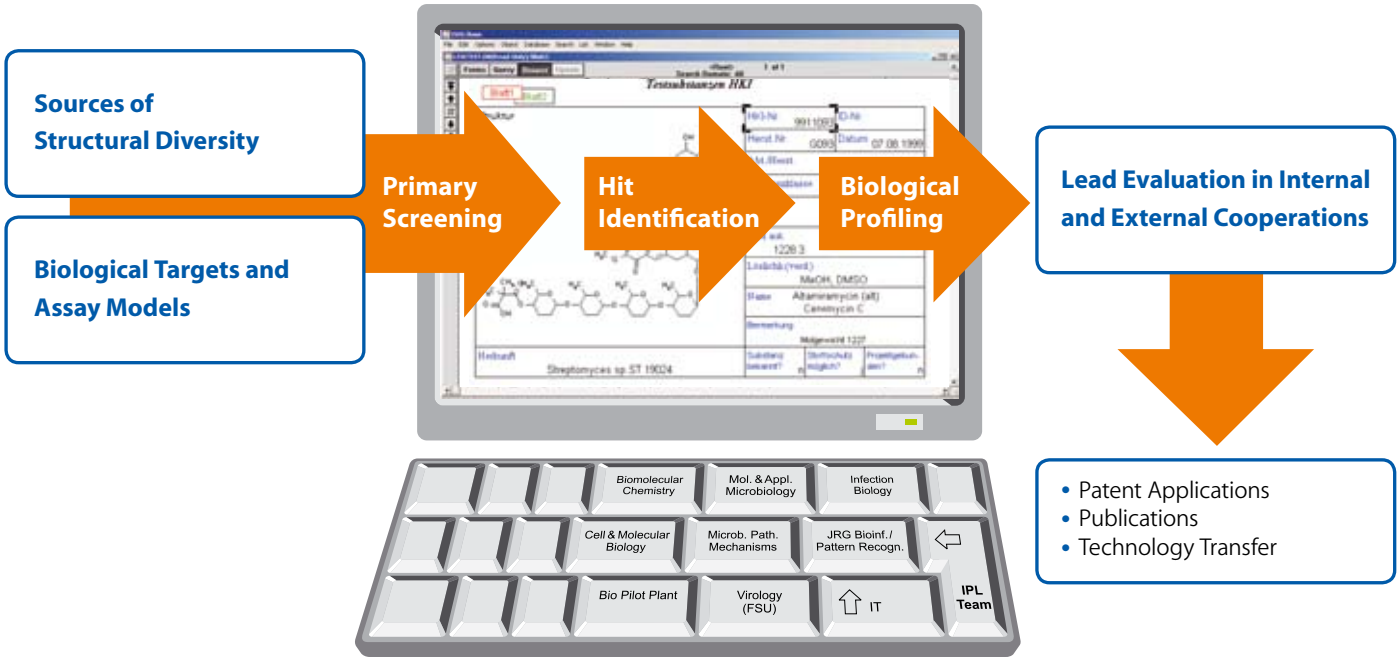
The assays established and available for identification and characterization of natural products include a diversity of biological communication systems. They facilitate studies of interactions between natural products and biological macromolecules. This implies molecular interactions of natural products with microorganisms but also with higher organisms and interference with complex pathogen/host-interactions.

stoffe 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
Biostructure Chemistry	Peter Gebhardt Kerstin Herold
Molecular Natural Products Research	Isabel Sattler (Deputy-Coordinator) Corinna Lange
Cell and Molecular Biology	Frank Hänel
Infection Biology	Ute Möllmann Uta Wohlfeld Hans-Martin Dahse
Molecular and Applied Microbiology	Michael Ramm Barbara Schütze
Pilot Plant for Natural Products	Karin Martin Martin Roth
Drug Testing	Albert Härtl
Administration	Reinald Schorcht Andrea Matthies
Institute of Virology and Antiviral Therapy, Friedrich Schiller University 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 more than 4.300 compounds. 287 compounds were newly added in 2004, 238 compounds in 2005. Additionally, the HKI maintains a natural products pool of 8.749 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 (established on ORACLE(TM)) is under development. 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 compounds

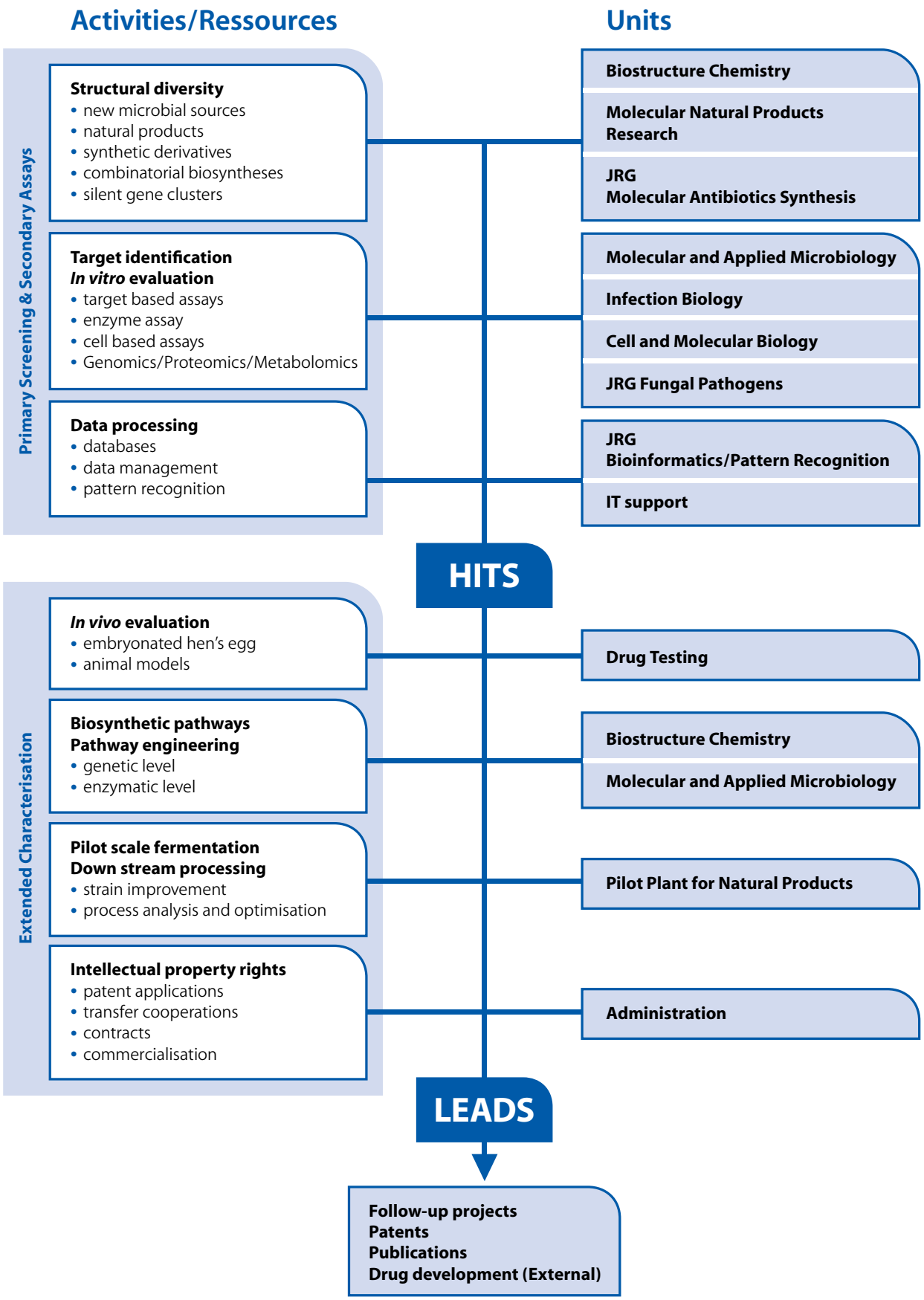
Cervimycin K is produced as a minor component by a *Streptomyces tendae* strain isolated from a cave with prehistoric rock paintings in Italy. The compound is active against problematic pathogens like multiresistant staphylococci and vancomycin resistant enterococci. The potentially new target and the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

In the extract of the filamentous fungus *Daedalea quercina* L.:F a **chromen** with exclusive antiinflammatoric activity was identified and isolated. A synthesis for a more easily accessible and novel **chroman** derivative with comparable activitiy was developed. Patents were filed for both, the biotechnological process and the synthesis. Both compounds were transferred to a company for application studies.

The new cyclodepsipeptides **lydiamycins A–C**, identified from *Streptomyces lydicus* by a physico-chemical screening approach, were shown to selectively inhibit fast growing species of mycobacteria without being cytotoxic. Further studies of the antibiotic activity of lydiamycin A against slow growing and pathogenic mycobacteria, including *M. tuberculosis* H37Rv (ATCC 25618) and a multiresistant clinical isolate (in collaboration with the Central Institute for Tuberculosis Research, Moscow) confirmed its anti-mycobacterial properties. Interestingly, the activity against the multiresistant strain of *M. tuberculosis* suggests a mechanism of action different from that of available therapeutics like isoniazid, rifampicin, etambutol and streptomycin.

The **dithiocarbamate** derivative E-144 has strong, selective *in vitro* activity against mycobacteria including multiresistant strains of *M. tuberculosis* with MIC's <0,25 µg/ml. *In vivo* therapeutic efficacy was demonstrated in a murine tuberculosis infection model. The compound is subject for target identification and for further chemical and biological studies within the EU project NM4TB and in close collaboration with a company.

Internal Product Line (IPL)
Durchgehende Bearbeitungslinie (DBL)



Network Fundamental Research

Network Fundamental Research
Netzwerk Grundlagenforschung

For the HKI, as member of the Leibniz Association, intensive networking between all departments and junior research groups, as well as with external partners is one of the key features. In 2003, the Network Fundamental Research was established to strengthen this positive development of interdisciplinary cooperation. After a competitive evaluation procedure attended by the scientific advisory board, those projects which open up 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 und Nachwuchsgruppen 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 Gundlagenforschung 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 nachfolgenden Tabellen geben einen Überblick über die bisher geförderten Projekte und die daran beteiligten Kooperationspartner.

Project number	Project leader Partners
2401	Michael Ramm Ute Möllmann Albrecht Berg
2402	Jürgen Wendland Alexander Tretiakov
2403	Frank Hänel Ernst Römer Isabel Sattler
2404	Albrecht Berg Albert Härtl Bettina Bardl Hans Krügel
2405	Christine Skerka Helmut Kasch
2406	Thomas Munder Peter Hortschansky
2407	Jürgen Wendland Raimund Eck Frank Hänel
2408	Volker Schroeckh Dirk Schwartz Karin Martin

2501	Michael Ramm Ute Möllmann Christian Hertweck Bernd Schneider
2502	Jürgen Wendland Hans Peter Saluz Ute Möllmann
2503	Anja Kunert Michael Ramm
2504	Ingrid Groth Erika Kothe Thomas Munder Isabel Sattler

Unit	Project	Time periode
Molecular and Applied Microbiology Infection Biology Biostructure Chemistry	Biosynthese von D-Rhamnan bei *Pseudomonas aeruginosa*: Kohlenhydrat modifizierende Enzyme als Targets für neuartige antiinfektive Wirkstoffe	05/2003 – 04/2004
Junior Research Group Fungal Pathogens Cell and Molecular Biology	Identifizierung von stressinduzierten Genen und Charakterisierung ihrer Rolle für die Virulenz von *Candida albicans*	04/2003 – 03/2004
Cell and Molecular Biology Molecular Natural Products Research Molecular Natural Products Research	Nortropin-Analoga als Inhibitoren der Wechselwirkung der Transkriptionsfaktoren Myc und Max	05/2003 – 04/2004
Biostructure Chemistry Drug Testing Pilot Plant for Natural Products Cell and Molecular Biology	Struktur, Wirkung und Biosynthesen peptidischer Naturstoffe	05/2003 – 04/2004
Infection Biology Biostructure Chemistry	Dreihybridsystem zur Identifikation von antifungalen Wirkstoffen	06/2003 – 06/2004
Cell and Molecular Biology Molecular and Applied Microbiology	Identifizierung und Charakterisierung viraler Pathogenitätsmechanismen durch Interaktionen multifunktioneller Coxsackievirus/Wirts-Proteinkomplexe	10/2003 – 09/2004
Junior Research Group Fungal Pathogens Infection Biology Cell and Molecular Biology	Zwei-Hybrid-Analysen von Proteinkomplexen innerhalb von Signalwegen, die an der Organisation des Zytoskeletts in *Candida albicans* beteiligt sind	06/2003 – 05/2004
Molecular and Applied Microbiology Junior Research Group Molecular Antibiotics Biosynthesis Pilot Plant for Natural Products	Bakterielle Wachstumsfaktoren	06/2003 – 06/2004

Molecular and Applied Microbiology Infection Biology Biostructure Chemistry Max Planck Institute for Chemical Ecology Jena	Auf der Suche nach neuen Antiinfektiva: Inhibitoren der Lipopolysaccharid-Biosynthese bei *Pseudomonas aeruginosa*	07/2004 – 12/2005
Growth-Control of Fungal Pathogens Cell and Molecular Biology 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 Molecular and Applied Microbiology	Immunevasion von *Pseudomonas aeruginosa*: Charakterisierung der Faktor-H-Bindung	11/2004 – 05/2006
Friedrich-Schiller-University Jena Cell and Molecular Biology Molecular Natural Products Research	Charakterisierung und Nutzung schwermetallresistenter und acidophiler Sporoactinomyceten: Molekulare Taxonomie, Proteomanalyse und Sekundärstoffwechselpotential	07/2004 – 12/2005

Project number	Coordinator Partners
2505	Christian Hertweck Wilhelm Boland Siegmund Reißmann Peter Zipfel
2506	Christian Hertweck Hans-Martin Dahse Martin Roth Ute Möllmann
2507	Kerstin Herold Christian Hertweck Peter Zipfel Hans Peter Saluz
2508	Peter Zipfel Christian Hertweck Kerstin Herold Hans Peter Saluz
2509	Hans Peter Saluz Christian Hertweck Kerstin Herold Peter Zipfel
2510	Peter Gebhardt Ute Möllmann Albert Härtl Hans-Martin Dahse
2511	Frank Hänel Christine Skerka
2512	Peter Zipfel Christian Hertweck
2513	Dirk Schwartz Ernst Roemer

Unit	Project	Time periode
Biostructure Chemistry Max Planck Institute for Chemical Ecology Jena Friedrich-Schiller-University Jena Infection Biology	Struktur- und Wirkungsanalyse von linearen und cyclischen Peptiden	07/2004 – 12/2005
Biostructure Chemistry Infection Biology Pilot Plant for Natural Products Infection Biology	Cervimycin-Projekt Teil A: Molekulare Grundlage der Cervimycin-Biosynthese – Funktionsanalyse und gerichtete Biosynthese von Cervimycin K und Analoga	10/2004 – 03/2006
Biostructure Chemistry Biostructure Chemistry Infection Biology Cell and Molecular Biology	Cervimycin-Projekt Teil B: Semisynthetische Darstellung des Cervimycin K und Bereitstellung neuer Antibiotika über chemische Modifikation der Cervimycine und Co-Fermentationsversuche, Untersuchungen zur Struktur-Wirkungsbeziehung	07/2004 – 12/2005
Infection Biology Biostructure Chemistry Biostructure Chemistry Cell and Molecular Biology	Cervimycin-Projekt Teil C: Wirkprofil und Wirktargets der Cervimycine mittels Proteomanalyse (Proteomics)	10/2004 – 03/2006
Cell and Molecular Biology Biostructure Chemistry Biostructure Chemistry Infection Biology	Cervimycin-Projekt Teil D: Wirkungsanalyse der Cervimycine auf humane Zellen	07/2004 – 12/2005
Biostructure Chemistry Infection Biology Drug Testing Infection Biology	Schutzgruppenfreie Semisynthese von Leucomycin-antibiotika mithilfe chemoselektiver Methoden	08/2004 – 12/2005
Cell and Molecular Biology Infection Biology	Rolle der Zinkfinger-Transkriptionsfaktoren MIZ-1 und EGR-1 bei der Regulation des Zellwachstums und der Apoptose	07/2004 – 12/2005
Infection Biology Biostructure Chemistry	Einsatz eines Protein-Microarrays zur Wirkstoff-Testung	12/2004 – 05/2006
Molecular Antibiotics Biosynthesis Molecular Natural Products Research	Untersuchungen zur Bereitstellung von aproteinogenen Aminosäuren in Antibiotikabiosynthesen	10/2004 – 03/2005

Appendix

Appendix

Peer Reviewed Articles 2004
Originalarbeiten 2004

Department Biostructure Chemistry

Berg A, **Wangun HV**, Nkengfack AE, **Schlegel B** (2004) Lignoren, a new sesquiterpenoid metabolite from *Trichoderma lignorum* HKI 0257. *J Basic Microbiol* 44, 317-319.

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He J, **Müller M**, **Hertweck C** (2004) Formation of the Aureothin Tetrahydrofuran Ring by a Bifunctional Cytochrome P450 Monooxygenase. *J Am Chem Soc* 126, 16742-16743.

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Hertweck C, Xiang L, Kalaitzis JA, Cheng Q, **Palzer M**, Moore BS (2004) Context-Dependent Behavior of the Enterocin Iterative Polyketide Synthase: A New Model for Ketoreduction. *Chem Biol* 11, 461-468.

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Kozlovsky AG, Zhelifonova VP, Antipova TV, Adanin VM, Novikova ND, Deshevaya EA, **Schlegel B**, **Dahse H-M**, **Gollmick F**, **Gräfe U** (2004) *Penicillium expansum*, a resident fungal strain of the orbital complex Mir, producing xanthocyllin X and questiomycin A. *Appl Biochem Microbiol* 40, 291-295.

Wangun HV, **Berg A**, **Hertel W**, Nkengfack AE, **Hertweck C** (2004) Anti-inflammatory and Anti-hyaluronate Lyase Activities of Lanostanoids from *Piptoporus betulinus*. *J Antibiot* 75, 755-758.

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Department Applied Microbiology

Brock M, Buckel W (2004) On the mechanism of action of the antifungal agent propionate, Propionyl-CoA inhibits glucose metabolism in *Aspergillus nidulans*. *Eu. J Biochem* 271, 3227-3241.

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Tüncher A, Reinke H, Martic G, Caruso ML, **Brakhage AA** (2004) A basic-region helix-loop-helix protein-encoding gene (devR) involved in the development of *Aspergillus nidulans*. *Mol Microbiol* 52, 227-241.

Zhang Y, **Brock M**, Keller N (2004) Connection of propionyl-CoA metabolism to polyketide biosynthesis in *Aspergillus nidulans*. *Genetics* 168, 785-794.

Each publication is listed in all departments and junior research groups from which the authors originate. In some cases this will lead to multiple citations.

Jede Publikation wird in den Abteilungen und Nachwuchsgruppen zitiert, aus denen die Autoren stammen. Dies führt in einigen Fällen zu Mehrfachnennungen.

Department Infection Biology

Cordes FS, Kraiczy P, Roversi P, **Skerka C**, Kirschfink M, Simon MM, Brade V, Lowe ED, **Zipfel PF**, Wallich R, Lea SM, (2004) Crystallization and preliminary crystallographic analysis of BbCRASP-1, a complement regulator acquiring surface protein of *Borrelia burgdorferi. Acta Crystallogr D Biol Crystallogr* 60, 929-932.

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Davydenko SG, Juselius JK, **Munder T**, Bogengruber E, Jantti J, Keranen S (2004) Screening for novel essential genes of *Saccharomyces cerevisiae* involved in protein secretion. *Yeast* 21, 463-471.

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Research Group Drug Testing

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Schwartz D, Berger S, Heinzelmann E, Muschko K, Welzel K, Wohlleben W (2004) The biosynthetic gene cluster of the herbicide phosphinothricin tripeptide from *Streptomyces viridochromogenes* Tü494. *Appl Environ Microbiol* 70, 7093-7102.

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Kühn AV, **Ozegowski JH**, **Peschel G**, Neubert R (2004) Complementary exploration of the action pattern of hyaluronate lyase from *Streptococcus agalactiae* using capillary electrophoresis, gel permeation chromatrography and viscosimetric measurements. *Carbohydr Res* 339, 2541-2547

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Department Biostructure Chemistry

Herold K, **Gollmick FA**, **Groth I**, **Möllmann U**, **Gräfe U**, **Hertweck C** (2005) Cervimycin A-D, a Polyketide Glycoside Complex from a Cave Bacterium Can Defeat Vancomycin Resistance. *Chem Eur J* 11, 5523-5530.

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

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

Wendland, Jürgen
Eukaryotic Cell, American Society for Microbiology, Washington DC
Journal of Basic Microbiology, Wiley-VCH, Weinheim
Microbiological Research, Elsevier, Jena

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Hammerschmidt, Sven (Zentrum für Infektionsforschung, Würzburg, Germany)
Interaction of *Streptococcus pneumoniae* with host proteins: adhesins and their binding motifs
20.01.2004
Host: Zipfel PF

Dudler, Robert (Institute of Plant Biology, University of Zurich, Switzerland)
Biosynthesis and action of Syringolin A, an unusual effector molecule produced by *Pseudomonas syringae*
18.02.2004
Host: Saluz HP

Zyma, Valentin Leonidovitsch (Department of Biophysics, University of Kiew, Ukraina)
Cell calcium signals: registration and quantitative estimation
22.06.2004
Host: Krügel H

Haas, Hubertus (Department of Molecular Biology, Innsbruck Medical University, Austria)
Siderophore-mediated iron uptake in *Aspergillus*
29.06.2004
Host: Zipfel PF

Józsi, Mihály (Dept. Infection Biology, HKI Jena, Germany)
Complement regulation at the cell surface: Multiple layers of defense
06.07.2004
Host: Zipfel PF

Schröder, Jens (Universitätsklinikum Schleswig-Holstein, Campus Kiel, Germany)
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13.07.2004
Host: Zipfel PF

Santoro, Raffaella (Deutsches Krebsforschungszentrum Heidelberg, Germany)
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Host: Saluz HP

Palm, Harry (Bogor Agricultural University, Bogor, Indonesia)
Systematische Untersuchungen an Rüsselbandwürmern und deren Rolle für das Verständnis der Evolution mariner fischparasitischer Helminthen
16.11.2004
Host: Saluz HP

Stachelhaus, Torsten (Fachbereich Chemie/Biochemie, Philipps-Universität Marburg, Germany)
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14.12.2004
Host: Zipfel PF

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Hoffmann, Ralf (Fakultät für Chemie und Mineralogie, Universität Leipzig, Germany)
Analyse posttranslationaler Proteinmodifikationen am Beispiel des Tau-Proteins
22.03.2005
Host: Zipfel PF

Zeng, An-Ping (Abteilung Genomanalyse, Gesellschaft für Biotechnologische Forschung Braunschweig, Germany)
Modeling of metabolic and genetic networks
19.04.2005
Host: Guthke R

Stocker, Reto (Universitätsspital Zürich, Switzerland)
Pathophysiology, monitoring and treatment of acute traumatic brain injury in humans: Facts and speculations, role of apoptosis?
10.05.2005
Host: Saluz HP

Simpson, Thomas (School of Chemistry, University of Bristol, UK)
Chemistry, enzymology and molecular genetics of polyketide biosynthesis
01.06.2005
Host: Hertweck C

Trauner, Dirk (Department of Chemistry, University of California, Berkeley, USA)
Streptomyces, Snails and Synthesis
28.06.2005
Host: Hertweck C

Laplace, Frank (Bundesministerium für Bildung und Forschung, Berlin, Germany)
Förderung der Genomforschung und Systembiologie durch das BMBF – Stand und Perspektiven.
16.08.2005
Host: Guthke R

Kurischko, Cornelia (Department of Animal Biology, University of Pennsylvania, Philadelphia, USA)
RAM: a conserved signaling network that regulates daughter-specific transcriptional activity, polarized morphogenesis and cell integrity in *Saccharomyces cerevisiae*
27.09.2005
Host: Saluz HP

HKI-Methodenkolloquium
04.10.2005
Host: Brakhage AA

Leslie, John (Department of Plant Pathology, Kansas State University, Manhattan, Kansas, USA)
Pathogenicity and molecular biology of the phytopathogenic fungi *Fusarium* and *Gibberella*
06.10.2005
Host: Brakhage AA

Leslie, John (Department of Plant Pathology, Kansas State University, Manhattan, Kansas, USA)
Workshop Scientific Writing
07.10.2005
Host: Brakhage AA

Holzhütter, Hermann-Georg (Institut für Biochemie, Medizinische Fakultät der Humboldt-Universität zu Berlin, Universitätsklinikum Charité, Berlin, Germany)
Analyse von Hochdurchsatzdaten auf der Basis von metabolischen Flussbilanz-Methoden
11.10.2005
Host: Guthke R

Koehl, Joerg (Cincinnati Children's Hospital Medical Center, Division of Molecular Immunology Cincinnati, Ohio, USA)
Crossroads between innate and adaptive immunity: The role of complement
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Host: Zipfel PF

Basse, Christoph (Max-Planck-Institut für terrestrische Mikrobiologie Marburg, Germany)
Mitochondrial virulence determinants and host dependent gene regulation in *Ustilago maydis*
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Host: Brakhage AA

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21.10.2005
Host: Scheibner O

HKI-Methodenkolloquium
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Host: Brakhage AA

Heesemann, Jürgen (Max von Pettenkofer-Institut für Hygiene und Medizinische Mikrobiologie der Ludwig-Maximilians-Universität München, Germany)
Über die immunmodulatorische Wirkung des *Yersinia* Virulenzantigens LcrV.
08.11.2005
Host: Zipfel PF

Marc, Janja (Faculty of Pharmacy, University of Ljubljana, Slovenia)
Basic aspects of molecular biology of osteoporosis
15.11.2005
Host: Saluz HP

Dahse, Hans-Martin (Dept. Infection Biology, HKI Jena, Germany)
Zytotoxizität und Phagocytose
22.11.2005

Krügel, Hans (Dept. Cell and Molecular Biology, HKI Jena, Germany)
Genotypisierung und Genexpression bei Prokaryonten
22.11.2005

Schroeckh, Volker (Dept. Molecular and Applied Microbiology, HKI Jena, Germany)
Sekundärmetabolit-Cluster in *Aspergillus nidulans* und *A. fumigatus*
22.11.2005

Homburg, Stefan (Institut für Molekulare Infektionsbiologie, Universität Würzburg, Germany)
A new pathogenicity island from *Escherichia coli* contains a pks gene cluster
05.12.2005
Host: Brakhage AA

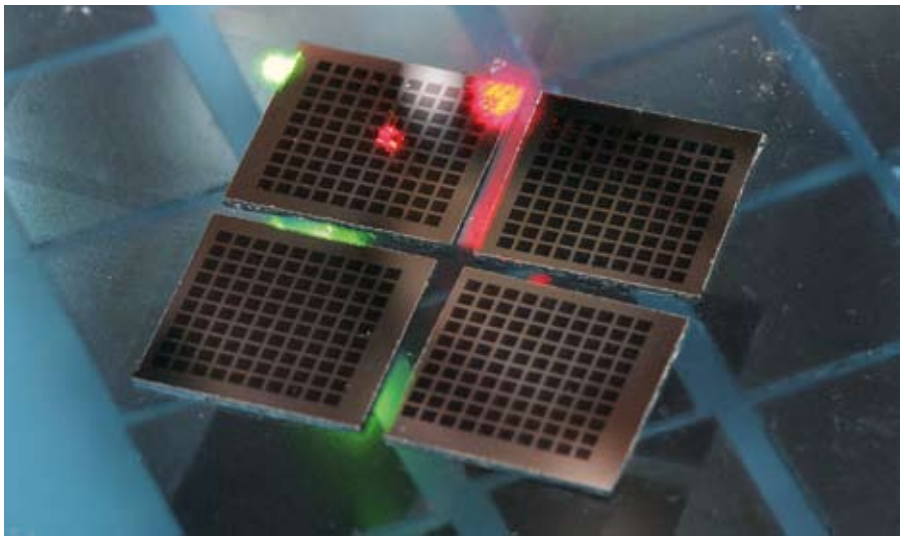
Schlünzen, Frank (Max-Planck-Institut für Molekulargenetik, Berlin, Germany)
Ribosomal antibiotics: Structural basis for the inhibition of protein synthesis
06.12.2005
Host: Hertweck C

Knorre, Wolfgang (HKI Jena, Germany)
1955–2005: Der Wandel unseres physikalischen Weltbildes (und wie ich ihn erlebt habe)
20.12.2005

Inventions and Patents 2004/2005
Erfindungen und Schutzrechte 2004/2005

Intellectual property rights as well as publications in peer reviewed journals are main performance parameters reflecting the quality of research at the HKI. In 2004/2005 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 cooperations with industry and affected the institute's budget advantageously. Two HKI scientists together with partners from three co-operating research institutions were honoured with the Thuringian Research Award in 2005 for a new technology involving microcultivation and investigation of microorganisms from single cells. The application for new patents is stringently evaluated in the HKI and focuses on new biologically active natural products and their synthetic derivatives. From 2006 the HKI will co-operate with Ascenion GmbH, Munich, to further optimize commerzialization activities. This project will be funded by the Federal Ministry of Education and Research.

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 2004/2005 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. Die in Kooperation mit drei anderen Forschungseinrichtungen entwickelte Technologie zur Mikrokultivierung und Untersuchung von Mikroorganismen aus Einzelzellen wurde mit dem Thüringer Forschungspreis 2005 ausgezeichnet. Die Anmeldung neuer Schutzrechte unterliegt einer strengen hausinternen Evaluation und wird sich verstärkt auf neue, biologisch aktive Naturstoffe und deren synthetische Derivate konzentrieren. In einem BMBF-geförderten Projekt zur effektiven Verwertung der Schutzrechte arbeitet das HKI ab 2006 mit dem externen Partner Ascenion GmbH, München zusammen.



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Scientific Awards 2004/2005
Preise und Auszeichnungen 2004/2005

He, Jing
medac-Forschungspreis
2004

Herold, Steffi
medac-Forschungspreis
2004

Jozsi, Mihaly
medac-Forschungspreis
2004

Wollmann, Yvonne
Meeting Fellowship Award of the 8th Meeting of the German DNA Repair Network, Ulm
2004

Zipfel, Peter
Thüringer Forschungspreis, Bereich Grundlagenforschung
2004

Brock, Matthias
Promotionspreis der Philipps-Universität Marburg
2005

He, Jing
Promotionspreis der Chinesischen Regierung
2005

Hertweck, Christian
DECHEMA Nachwuchswissenschaftler-Preis für Naturstoff-Forschung
2005

Hertweck, Christian
Wissenschaftspreis „Lebenswissenschaften und Physik“ des Beutenberg-Campus Jena 2004
verliehen am 10.11.2005

Hertweck, Christian
Thüringer Forschungspreis, Bereich Grundlagenforschung
2005

Hortschansky, Peter
Themistocles GLUCK-Preis für Endoprothetik der Deutschen Gesellschaft für Orthopädie und Orthopädischen Chirurgie e. V.,
2005

Martin, Karin
Thüringer Forschungspreis, Bereich Angewandte Forschung
2005

Partida-Martinez, Laila Pamela
Thüringer Forschungspreis, Bereich Grundlagenforschung
2005

Roth, Martin
Thüringer Forschungspreis, Bereich Angewandte Forschung
2005

Walther, Andrea
Wissenschaftspreis „Lebenswissenschaften und Physik“ des Beutenberg-Campus Jena 2005
verliehen am 20.04.2006

Walther, Andrea
Promotionspreis der Friedrich-Schiller-Universität Jena für die Dissertation
2005
verliehen am 30.06.2006

Meetings, Workshops, Symposia 2004/2005
Wissenschaftliche Veranstaltungen 2004/2005

2nd Spring School and Workshop on Intelligent Technologies for Gene Expression Based Individualized Medicine
Pfaff M, **Guthke R**
Jena, Germany
May 2004

VAAM: International Meeting on the Biology of Bacteria Producing Natural Products
Hertweck C, Piel J, **Schwartz D**
Jena, Germany
September 2004

Statusworkshop der DGHM-Fachgruppe „Eukaryontische Krankheitserreger“
Zipfel PF
Jena, Germany
Februar 2005

2nd International Workshop „Thrombotic Mikroangiopathies“
Zipfel PF
Jena, Germany
May 2005

SPP 1160 Schwerpunkttagung “Colonisation and infection by human-pathogenic fungi”
Brakhage AA
Jena, Germany
Februar 2005

Spring School and Workshop on Reverse Engineering in Systems Biology
Pfaff M, **Guthke R**
Jena, Germany
June 2005

Workshop on Nature-inspired Modeling, Optimization and Control
Guthke R, Westra R
Albufeira, Portugal
October 2005

Participation in Research Networks 2004/2005
Beteiligung an Netzwerken und Verbundprojekten 2004/2005

Sonderforschungsbereiche der Deutschen Forschungsgemeinschaft

Sonderforschungsbereich 604: Multifunktionelle Signalproteine
Teilprojekt B1: Multiple functions of the phosphatidylinositol-3-kinase Vps34p of the human pathogenic yeast *Candida albicans*
Uwe Horn
Laufzeit: 01/2002 – 12/2004

Sonderforschungsbereich 604: Multifunktionelle Signalproteine
Teilprojekt B2: 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 1111: Zellpolarität
Teilprojekt: Analyses on the role of *RHO3* and of the WASP-homologue *WAL1* for the maintenance of polarized hyphal growth in *Candida albicans*.
Jürgen Wendland
Laufzeit: 11/2003 – 10/2005

Schwerpunktprogramm 1150: Wege zum Aktinzytoskeletts 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 I: 08/2003 – 07/2005
Laufzeit II: 08/2005 – 12/2006

Schwerpunktprogramm SPP 1152: Evolution metabolischer Diversität
Teilprojekt: Evolution and function of cis-/trans elements of fungal secondary metabolism with emphasis on the penicillin biosynthesis
Axel A. Brakhage
Laufzeit: 05/2003 – 05/2005

Schwerpunktprogramm 1160: Kolonisation und Infektion durch humanpathogene Pilze
Koordination
Axel A. Brakhage
Laufzeit 2004 – 2010

Schwerpunktprogramm 1160: Kolonisation und Infektion durch humanpathogene Pilze
Teilprojekt: Identification of virulence determinants of the human-pathogenic fungus *Aspergillus fumigatus* by proteome analysis
Axel A. Brakhage
Laufzeit: 02/2005 – 08/2007

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: 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: Immune and complement evasion of *Candida albicans*
Peter F. Zipfel
Laufzeit: 05/2004 – 04/2006

Verbundprojekte des Bundesministeriums für Bildung und Forschung

Kompetenznetzwerk: Genomforschung an Bakterien für den Umweltschutz, die Landwirtschaft und die Biotechnologie
Teilprojekt: Genetik der Biosynthese gemischter Sekundär-Metabolite in Streptomyceten
Christian Hertweck / Dirk Schwartz
Laufzeit: 06/2001 – 05/2004

Kompetenznetzwerk: Genomforschung an Bakterien für den Umweltschutz, die Landwirtschaft und die Biotechnologie
Teilprojekt: Analyse der Biosynthese gemischter Sekundärmetabolite in Streptomyceten
Christian Hertweck/Dirk Schwartz
Laufzeit: 06/2004 – 08/2006

Verbundprojekt: BIO-Instrumente Jena
Teilprojekt: Diagnose von infektionsbedingten Entzündungsreaktionen (Sepsis) mittels DNA- bzw. Protein-Array Technologie
Peter F. Zipfel/Hans Peter Saluz
Laufzeit: 04/2001 – 03/2004

Verbundprojekt BioChancePLUS-2: 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

Verbundprojekt des Umweltbundesamtes: Untersuchungen zum Vorkommen und zur gesundheitlichen Relevanz von Bakterien in Innenräumen
Teilprojekt: Streptomyceten in Innenräumen
Ingrid Groth
Laufzeit: 12/2005 – 05/2008

Verbundprojekt 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

Verbundprojekt Knochenersatzmaterialien zur Therapie der Osteoporose
Peter Hortschansky
Laufzeit: 07/2004 – 06/2007

Verbundprojekt Mikroreaktorik für High Throughput-Einzelzellkulturen von Mikroorganismen – MINIKULT
Teilvorhaben: Systemevaluierung
Martin Roth
Laufzeit: 10/2001 – 12/2004

Verbundprojekt Hochdurchsatz-Bioassay-System auf Basis mikroserieller Zellkulturen in flüssig/flüssig-Zweiphasensystemen – SERIZELL
Teilvorhaben: Systemevaluierung mit ausgewählten Bioassays
Martin Roth
Laufzeit: 01/2005 – 12/2007

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

Schwerpunkt: Quality of Life and Management of Living Resources (FP5)
Teilprojekt: Structural and Functional Genomics of *Mycobacterium tuberculosis*
Ute Möllmann
Laufzeit: 10/2002 – 12/2005

Marie Curie Research Training Networks SIGNALPATH (FP6)
MAP kinase cascades controlling virulence in fungi: from signals to pathogenicity response
Axel A. Brakhage
Laufzeit: 11/2005 – 0/2009

Integrated Project: New medicines for tuberculosis (NM4TB) (FP6)
Ute Möllmann
Laufzeit: 01/2007 – 12/2008

Schwerpunkt: Information Society Technologies (FP6)
Teilprojekt: Nature-inspired Smart Information Systems
Reinhard Guthke
Laufzeit: 01/2005 – 01/2008

Integrated Project: European Network on Intelligent Technologies for Smart Adaptive Systems (EUNITE) (FP6)
Teilprojekt: Intelligent technologies for gene expression based individualised medicine
Reinhard Guthke
Laufzeit: 07/2002 – 06/2005

Academic Teaching 2004/2005
Lehre 2004/2005

Brakhage, Axel A.
Brock, Matthias
Groth, Ingrid
Heinekamp, Thorsten
Hortschansky, Peter
Kniemeyer, Olaf
Schroeckh, Volker
Lecture, Seminar, Practical course
Molekulare Biologie und Genetik der Pilze
Friedrich-Schiller-Universität Jena

Brakhage, Axel A.
Brock, Matthias
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Lecture, Seminar, Practical course
Angewandte Mikrobiologie
Friedrich-Schiller-Universität Jena

Grabley, Susanne
Groth, Ingrid
Munder, Thomas
Sattler, Isabel
Schwartz, Dirk
Lecture, Seminar, Practical course
Biochemische Analytik
Friedrich-Schiller-Universität Jena

Knorre, Wolfgang A.
Christner, Arnulf
Guthke, Reinhard
Horn, Uwe
Lecture, Seminar, Practical course
Biotechnologie/Bioverfahrenstechnik
Friedrich-Schiller-Universität Jena

Saluz, Hans Peter
Hänel, Frank
Lecture, Seminar, Practical course
Molekulare Genetik
Friedrich-Schiller-Universität Jena

Zipfel, Peter F.
Hellwage, Jens
Skerka, Christine
Lecture, Seminar, Practical course
Molekulare Biologie/Immunologie
Friedrich-Schiller-Universität Jena

Hertweck, Christian
Gebhardt, Peter
Gollmick, Friedrich
Schwartz, Dirk
Perner, Andrea
Lecture, Seminar, Practical course
Naturstoffchemie
Friedrich-Schiller-Universität Jena

Hertweck, Christian
Gebhardt, Peter
Gollmick, Friedrich
Schwartz, Dirk
Perner, Andrea
Lecture, Seminar, Practical course
Molekulare Grundlagen des bakteriellen Sekundärmetabolismus
Friedrich-Schiller-Universität Jena

Guthke, Reinhard
Möller, Ulrich
Lecture, Seminar, Practical course
Bioinformatik
Friedrich-Schiller-Universität Jena

Guthke, Reinhard
Möller, Ulrich
Lecture, Seminar, Practical course
Analyse der Genexpression
Friedrich-Schiller-Universität Jena

Munder, Thomas
Lecture, Seminar, Practical course
Genregulation/Entwicklung
Friedrich-Schiller-Universität Jena

Hellwage, Jens
Schwartz, Dirk
Lecture, Seminar, Practical course
Molekulare Biologie
Friedrich-Schiller-Universität Jena

Wendland, Jürgen
Lecture, Seminar, Practical course
Mikrobiologie für Ernährungswissenschaftler
Friedrich-Schiller-Universität Jena

Ramm, Michael
Lecture, Seminar, Practical course
Pharmazeutische und Medizinische Mikrobiologie
Friedrich-Schiller-Universität Jena

Christner, Arnulf
Horn, Uwe
Lecture
Bioverfahrenstechnik/Pharmaka
Fachhochschule Jena

Calls for Appointments 2004/2005
Rufe 2004/2005

Brakhage, Axel A. (2004)
Lehrstuhl für Mikrobiologie und Molekularbiologie
Biologisch-Pharmazeutische Fakultät der Friedrich-Schiller-Universität Jena

Schwartz, Dirk (2005)
Professor für Molekularbiologie und Bioanalytik
Hochschule Esslingen

Postdoctoral Lecture Qualifications 2004/2005
Habilitationen 2004/2005

Wendland, Jürgen (2004)
Molekulare Analysen zum polaren Hyphenwachstum und zur Septierung bei *Ashbya gossypii* und *Candida albicans*
Friedrich-Schiller-Universität Jena

Hertweck, Christian (2005)
Über das Verständnis der mikrobiellen Polyketidbiosynthese zu neuen Wirkstoffen
Friedrich-Schiller-Universität Jena

Graduations 2004/2005
Promotionen 2004/2005

2004

Guan, Shuhong
Secondary metabolites from endophytes of mangrove plant *Kandelia candel* (L.) Druce
Peking University

Günther, Juliane
Generierung und funktionelle *in vivo* Charakterisierung einer Lipidkinase-defekten Phosphatidylinositol 3-Kinase CaVps34p der humanpathogenen Hefe *Candida albicans*
Friedrich-Schiller-Universität Jena

Günther, Sebastian
DNA microarray technology for the classification of the actinomycetes *Kitasatospora*
Friedrich-Schiller-Universität Jena

Limmon, Gino
Gene Expression Profile and Apoptosis Inhibition mechanisms in *Chlamydia pneumoniae* infected cells
Friedrich-Schiller-Universität Jena

Majno, Sandra
Funktionelle Charakterisierung des Faktor H-verwandten Proteins FHR-1 im Komplementsystem
Friedrich-Schiller-Universität Jena

2005

Eminger, Daniela
Molekulargenetische Analyse der Biosynthese von Antibiotika mit Polyketid-Peptid Mischstrukturen (H)
Friedrich-Schiller-Universität Jena

Fritzsche (Jakobi), Kathrin
Kombinatorische Biosynthese von aromatischen Polyketiden
Friedrich-Schiller-Universität Jena

Han, Li
Secondary metabolites from mangrove plants *Avicennia marina* and *Bruguiera gymnorrhiza* and their related microbionts
Peking University

He, Jing
Molecular analysis of the aureothin biosynthesis gene cluster
Friedrich-Schiller-Universität Jena

Herold, Kerstin
Untersuchungen zur Struktur, Wirkungsweise und Biosynthese des Cervimycins als besondere Klasse aromatischer Polyketide aus Streptomyceten
Friedrich-Schiller-Universität Jena

Li, Liya
Secondary metabolites from mangrove plant *Hibiscus tiliaceus* and its related microbionts
Peking University

Loos, Sabine
Expression eukaryotischer PKS in heterologen Wirten
Friedrich-Schiller-Universität Jena

Ludajic, Katarina
Functional characterization of Early Growth Response Transcription Factor 2 (EGR-2)
Friedrich-Schiller-Universität Jena

Monossov, Vladimir
Discovery and Validation of Rule-Based Knowledge by Gene Expression Analysis
Friedrich-Schiller-Universität Jena

Müller, Claudia
Analyse der Biosynthese des Lipopeptidantibiotikums Friulimicin in *Actinoplanes friulienses* (H)
Friedrich-Schiller-Universität Jena

Ruryk, Andriy
Development of microsystem technology suitable for bacterial identification and gene expression monitoring.
Friedrich-Schiller-Universität Jena

Walther, Andrea
Analyse G-Protein vermittelter Signale auf die Organisation des Aktinzytoskeletts und das polare Wachstum von *Candida albicans* und *Ashbya gossypii*
Friedrich-Schiller-Universität Jena

Wollmann, Yvonne
Das Miz-1 interagierende Protein TOP BP1: Identifizierung neuer Interaktionspartner und Untersuchungen zur Rolle bei der Zellzyklusregulation.
Friedrich-Schiller-Universität Jena

Diploma Theses 2004/2005
Diplomarbeiten 2004/2005

2004

Erhardt, Annette
Untersuchungen zur Apoptose-Inhibition in menschlichen Fibroblasten nach Infektion mit *Chlamydia pneumoniae*

Güttich, Sven
Generierung und Charakterisierung einer vollsynthetischen Camelidae-VHH-Antikörper-Bibliothek in *Escherichia coli*

Hausdorf, Lena
Untersuchungen zur Bereitstellung von aproteinogenen Aminosäuren in Antibiotikabiosynthesen

Hellwig, Daniela
Untersuchung der Kolonisierungsfähigkeit von *Candida albicans* Mutanten auf Schweinedarmepithel und Funktionsanalyse von *CaEDE1*

Krebs, Franziska
Suche und Charakterisierung neuer Protein-Protein-Interaktionen in apoptotischen Prozessen

Palzer, Michelle
Molekularbiologische Untersuchung der Biosynthese der Polyketidglykoside Cervimycin und Chartreusin in Streptomyceten

Popp, Anke
Wirkung von Doxycyclin auf *Chlamydia pneumoniae* infizierte humane Zellen mit dem Fokus auf apoptotische Prozesse

Rappl, Anne
Beeinflussung von apoptotischen Prozessen in humanen Tumorzellen durch Bestrahlung mit UVC Licht

Reinert, Susanne
Synthese eines Heterodimeren für ein 3-Hybridsystem

Schenk, Tino
Apoptose und differentielle Genexpression in mit humanen Papillomaviren infizierten Zervixkarzinomzelllinien

Schümann, Julia
Untersuchungen zur molekularen Basis der Cytochalasin-Biosynthese in Pilzen

Schwalbe, Yvonne C.N
Konstruktion und Screening einer *Candida albicans* Zwei-Hybrid-cDNA-Genbank

Ziehl, Martina
Studien zur Mutasynthese neuer Aureothin-Derivate

Zöllner, Tina
Chemische Derivatisierung von 16-gliedrigen Makroliden über selektive Oxidation und Methathese

2005

Bock, Katrin
Lokalisierung und funktionelle Charakterisierung der EGR-Zinkfingerproteine in T-Zellen

Busch, Benjamin
Funktionsuntersuchungen einer iterativen Typ I Polyketidsynthase aus *Streptomyces thioluteus* HKI-227

Ebbinghaus, Matthias
Untersuchungen zur Interaktion zwischen viralen und zellulären Proteinen: Implikationen für die Pathogenese von Coxsackievirus B3

Fleck, Christian
Identification and characterisation of an Acyl-CoA: acylate CoA-transferase from *A. nidulans*

Gruszin, Christin
Identifizierung und Charakterisierung Faktor-H-bindender Proteine aus *Pseudomonas aeruginosa*

Jezewski, Susann
Funktionelle Charakterisierung des bifunktionalen Enzyms der *de novo* Purin-Biosynthese Ade5,7p in der humanpathogenen Hefe *Candida albicans*

Jünemann, Katrin
Klonierung und Charakterisierung des Promotors der humanen mitogen-aktivierten Proteinkinase p38α

Kurt, Juliane
Interaktion zwischen den Mitgliedern der 'Early Growth Response' (EGR) Proteinfamilie und dem Tumorsuppressorprotein p53

Lauer, Nadine
Funktionelle Bedeutung der bei der altersabhängigen Maculadegeneration des Auges beschriebenen Faktor H bzw. FHL-1 Mutation H402Y

Poltermann, Sophia
Funktionelle Charakterisierung der vakuolären ATPase-Untereinheit Vma7p aus *Candida albicans*

Richter, Martin
Untersuchungen zur Substratspezifität von AurF, einer nitrobildenden N-Oxygenase aus *Streptomyces thioluteus*

Schindler, Stefanie
Funktionelle Charakterisierung des Promotors der humanen MAP Kinase p38α durch Reportergen-Analysen und 2D-Gelelektrophorese.

Schmidt, Uta
Die Inhibition der Wirtszellapoptose durch Chlamydien. Charakterisierung eines chlamydialen 27 kDa-Proteins.

Schultz, Julia
Interaction of Taf14 with TFIIS in *Saccharomyces cerevisiae*: a study of regulation of transcript elongation

Steinbach, Yvonne
Selektion von Phosphorylierungstargets von eukaryotischen Kinasen mittels der Peptide-on-Plasmid-Technologie

Stengel, Sven
Untersuchungen zur Expression und Regulation des humanen Tumormarkers PRAME

Strohbusch, Maria
Phosphatidylinositol 3-Kinase Vps34p von *Candida albicans* – Charakterisierung von Interaktionspartnern –

Wünsche, Hendrik Dezember
Comperative proteome analysis of herbivore-induced *Nicotiana attenuata* leaves

External Funding 2004/2005
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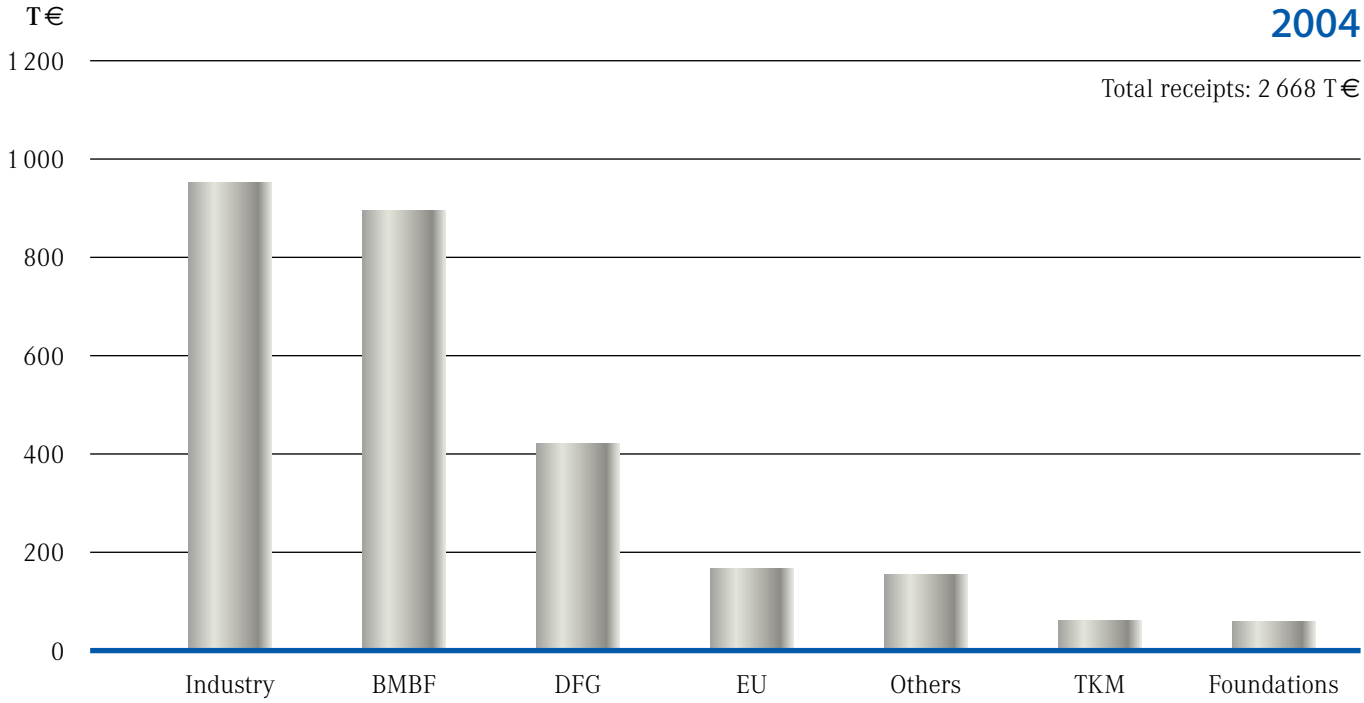

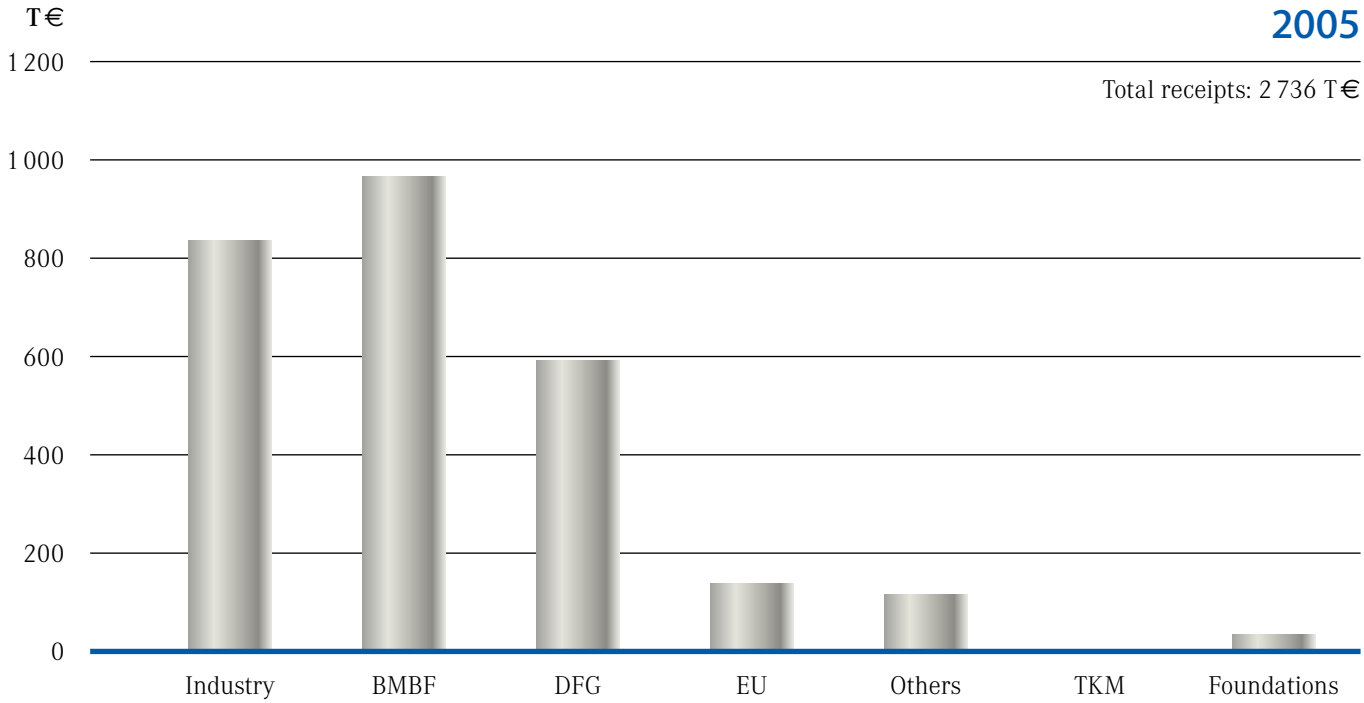

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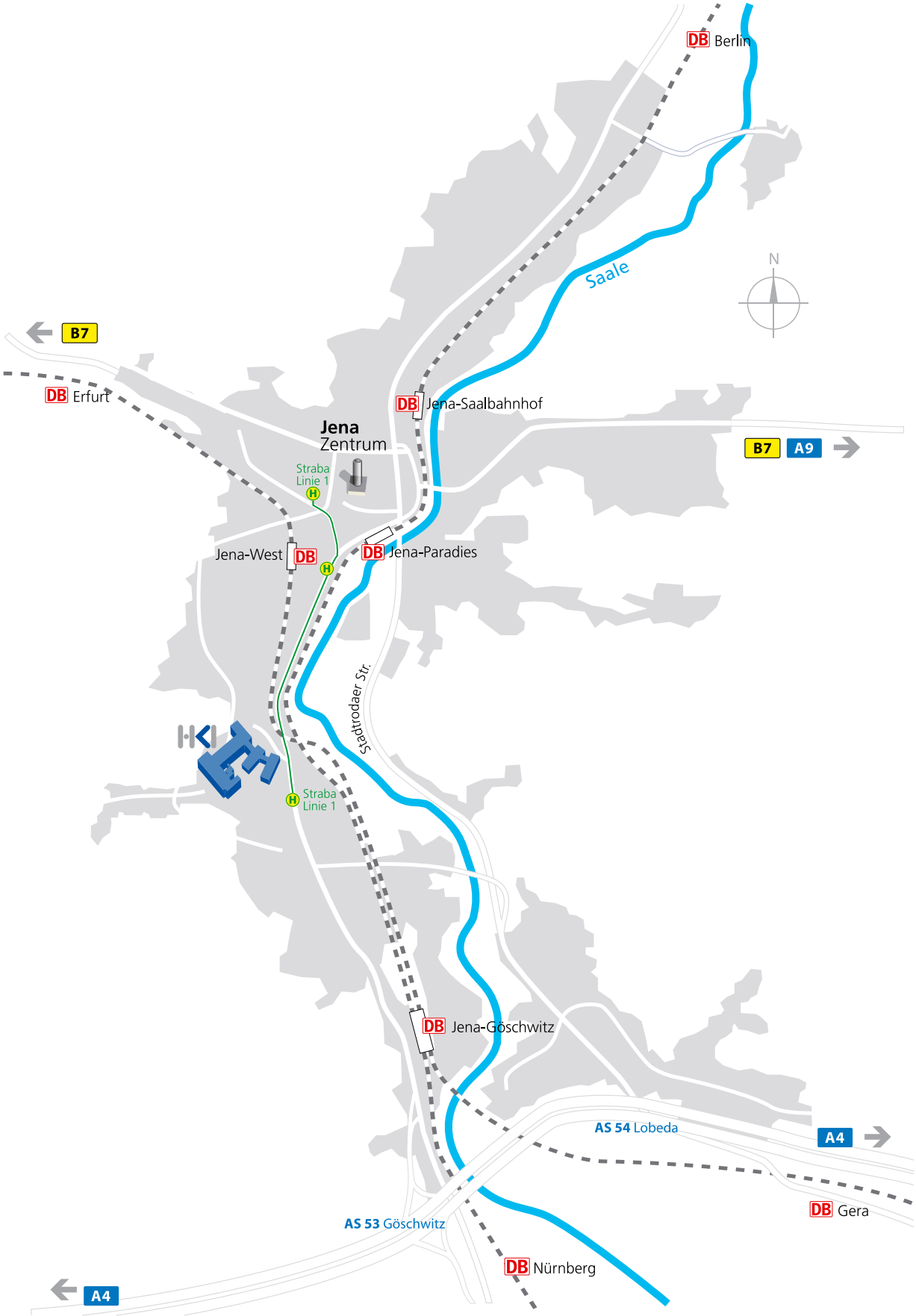

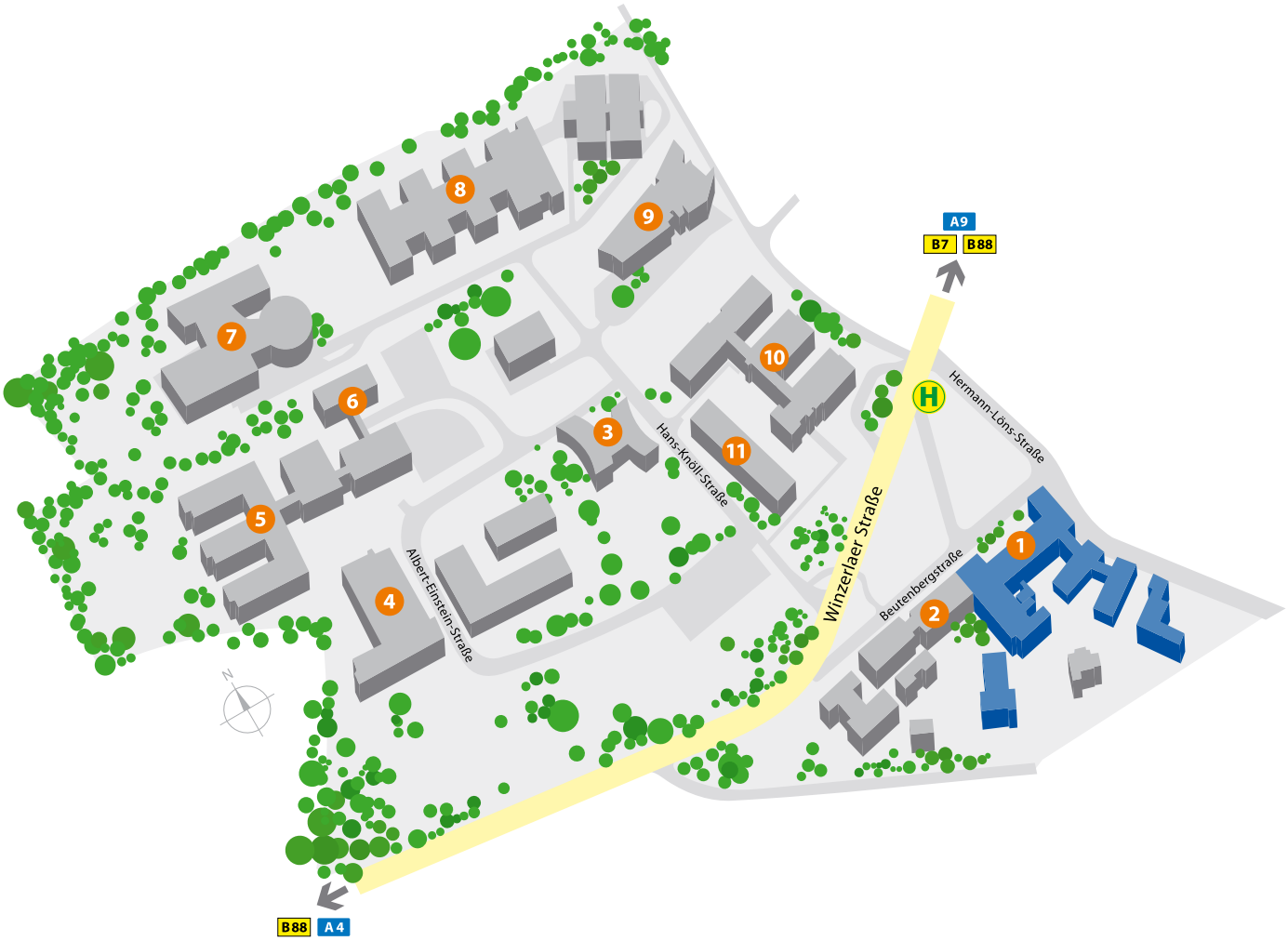

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