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

Research Report 2008 | 2009

The Leibniz Institute for Natural Product Research and Infection Biology – Hans Knöll Institute (HKI) – has made a considerable development in the past two calendar years. Of major importance was the publication of the results of the institute's evaluation in June 2007 by the Leibniz Association. The Institute was evaluated between very good and excellent. This impressive success encouraged us to further follow our scientific direction that also helps to develop a distinct scientific profile in Jena. The most important project was the establishment and further development of the excellence graduate school "*Jena School for Microbial Communication*", that was established at the Friedrich Schiller University Jena in close collaboration with the HKI and the MPI for chemical Ecology which offers an umbrella for all scientists from all over Jena irrespective of their institutional basis. Also, an important contribution to the JSMC was made by our Leibniz graduate school "International Leibniz Research School for Microbial and Biomolecular Interactions Jena". Another milestone for the Institute was the successful application for a *Centre for Innovation Competence "Septomics"* that has been established together with Prof. Konrad Reinhart (Clinic for Anaesthesiology and Intensive Therapy, University Hospital Jena) and Prof. Eberhard Straube (Institute for Medical Microbiology, University Hospital Jena). This centre is intended to link basic research and clinical research in Jena in the field of sepsis. It is a joint application of the Friedrich Schiller University, the university hospital and the HKI. Prof. Oliver Kurzai from Würzburg accepted the call for the professorship *"Fungal Septomics"*

INTRODUCTION | VORWORT

Das Leibniz-Institut für Naturstoff-Forschung und Infektionsbiologie – Hans-Knöll-Institut (HKI) – hat auch in den letzten beiden Kalenderjahren eine stürmische Entwicklung genommen. Von größter Bedeutung war die Veröffentlichung des Bewertungsberichts des HKI durch die Leibniz-Gemeinschaft, der das HKI als sehr gut bis exzellent ausweist. Basierend auf diesem großartigen Erfolg konnten wissenschaftliche und strukturelle Weiterentwicklungen in Angriff genommen werden, die insgesamt auch zur Profilbildung des Wissenschaftsstandorts Jena beitragen. Das wichtigste Projekt in diesem Zusammenhang war die Etablierung und der Ausbau der Exzellenz-Graduiertenschule *„Jena School for Microbial Communication"*, die durch die Friedrich-Schiller-Universität Jena in enger Kooperation mit dem HKI und dem MPI für chemische Ökologie erfolgreich eingeworben werden konnte und die Wissenschaftler in Jena über alle institutionellen Grenzen hinweg zusammenführt. Wesentlicher Bestandteil der JSMC ist unsere Leibniz-Graduiertenschule *„International Leibniz Research School for Microbial and Biomolecular Interactions Jena"*, die sich inzwischen fest etabliert hat.

Ein weiterer Meilenstein für das Institut war die erfolgreiche Einwerbung des Zentrums für Innovationskompetenz „ZIK Septomics" zusammen mit Prof. Konrad Reinhart (Klinik für Anästhesiologie und Intensivtherapie, Universitätsklinikum Jena) und Prof. Eberhard Straube (Institut für Medizinische Mikrobiologie, Universitätsklinikum Jena). Dieses Zentrum wird im Bereich Sepsis die Grundlagenforschung am HKI und Instituten des Universitätsklinikums mit der klinischen Forschung in Jena verknüpfen. Prof. Oliver Kurzai konnte

in this centre. Furthermore, the HKI contributed to the successful application for an integrated research and treatment center (*"Center for Sepsis Control and Care"*) at the University Hospital that further strengthens the focus on sepsis and invasive fungal infections in Jena. We were glad to welcome at the HKI Dr. Peter Staib from Lausanne and Dr. Markus Nett from San Diego as heads of the independent Junior Research Groups "Fundamental Molecular Biology of Pathogenic Fungi" and "Secondary Metabolism of Predatory Bacteria", respectively. Furthermore, a number of new projects were granted to the HKI, such as the project *"Genomics-Guided Discovery of New Antimicrobials using Microfluidic Devices"*. In this project, in collaboration with the Institute for Photonic Technologies (IPHT), the classical research topic of the HKI, i.e., the search for new drugs, is combined with the application of novel microfluidic devices. The high quality of the Institute's research is also documented by publications in Journals with a high impact factor such as "Nature", "Nature Chemical Biology", "Science", "Nature Biotechnology", "PLoS Pathogens", "PNAS" or "Angewandte Chemie International Edition", to mention a few. There is a close connection between this high quality research and the positive number of awards and prizes which were won by employees of the HKI in the reporting period. The activities of the HKI also find their expression in the organization of numerous meetings and conferences. This also includes the application colloquium in the framework of the DFG priority programme 1160 "Colonisation and Infection by human-pathogenic fungi".

als einer der beiden Gruppenleiter gewonnen werden. In einer gemeinsamen Berufung von FSU Jena mit dem HKI wurde er auf die Professur Fungal Septomics berufen. Das HKI konnte auch zur Einwerbung eines Integrierten Forschungs- und Behandlungszentrums („*Center for Sepsis Control and Care*") beitragen, wodurch der Schwerpunkt Sepsis und invasive Pilzinfektionen in Jena wesentlich gestärkt wurde.

Im personellen Bereich konnte Dr. Peter Staib – vorher in Lausanne tätig – für die Leitung der Nachwuchsgruppe „Molekularbiologische Grundlagen pathogener Pilze" gewonnen werden. Dr. Markus Nett folgte dem Ruf auf die Leitung der Nachwuchsgruppe „Sekundärmetabolismus räuberischer Bakterien" am HKI aus San Diego. Viele weitere Projekte bereichern die schon vorhandenen Forschungsarbeiten des Instituts, wie zum Beispiel das Vorhaben „*Genomics-Guided Discovery of New Antimicrobials using Microfluidic Devices*", in dem ein klassischer Forschungsschwerpunkt des HKI – die Suche nach neuen Wirkstoffen – mit der Anwendung neuester mikrofluidischer Technologien in Kooperation mit dem Institut für Photonische Technologien Jena verbunden wird. Die hohe Qualität der Forschung des Hans-Knöll-Instituts äußert sich auch in Publikationen in den renommiertesten Zeitschriften, wie „Nature", „Nature Chemical Biology", „Science", „Nature biotechnology", „PLoS Pathogens", „PNAS" oder „Angewandte Chemie International Edition", um nur einige zu nennen. Zusammenhängend mit dieser qualitativ hochwertigen Forschung ist die erfreuliche Zahl von Preisen, die im Berichtszeitraum an Mitarbeiter des Hans-Knöll-Instituts überreicht wurden. Die

I would like to thank all of my colleagues at the HKI. Only on the basis of intensive team work it is possible to maintain and increase the high level of science and application we have reached. I would also like to thank all employees for helping to write this report 2008/2009, in particular Dr. Michael Ramm.

I would be glad if you enjoyed reading this report.

Jena, June 2010

Axel Brakhage
Director of the HKI

Aktivitäten des Hans-Knöll-Instituts äußern sich nicht zuletzt auch in der Ausrichtung einer großen Zahl von Tagungen und Kolloquien, z. B. das Antragskolloquium des DFG-Schwerpunktprogramms 1160 „Kolonisation und Infektion durch human-pathogene Pilze“.

Für die ausgezeichnete Kooperation möchte ich mich bei allen Kolleginnen und Kollegen des HKI sehr herzlich bedanken. Nur durch die hervorragende Teamarbeit ist es möglich, die hohe Qualität der wissenschaftlichen Leistung des HKI zu halten und weiter zu steigern. Für die Mitarbeit an diesem Report 2008/2009 danke ich allen Mitarbeitern des HKI, insbesondere Dr. Michael Ramm.

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

Jena, im Juni 2010

Prof. Dr. Axel A. Brakhage
Direktor des HKI

HKI Research Report 2008 | 2009

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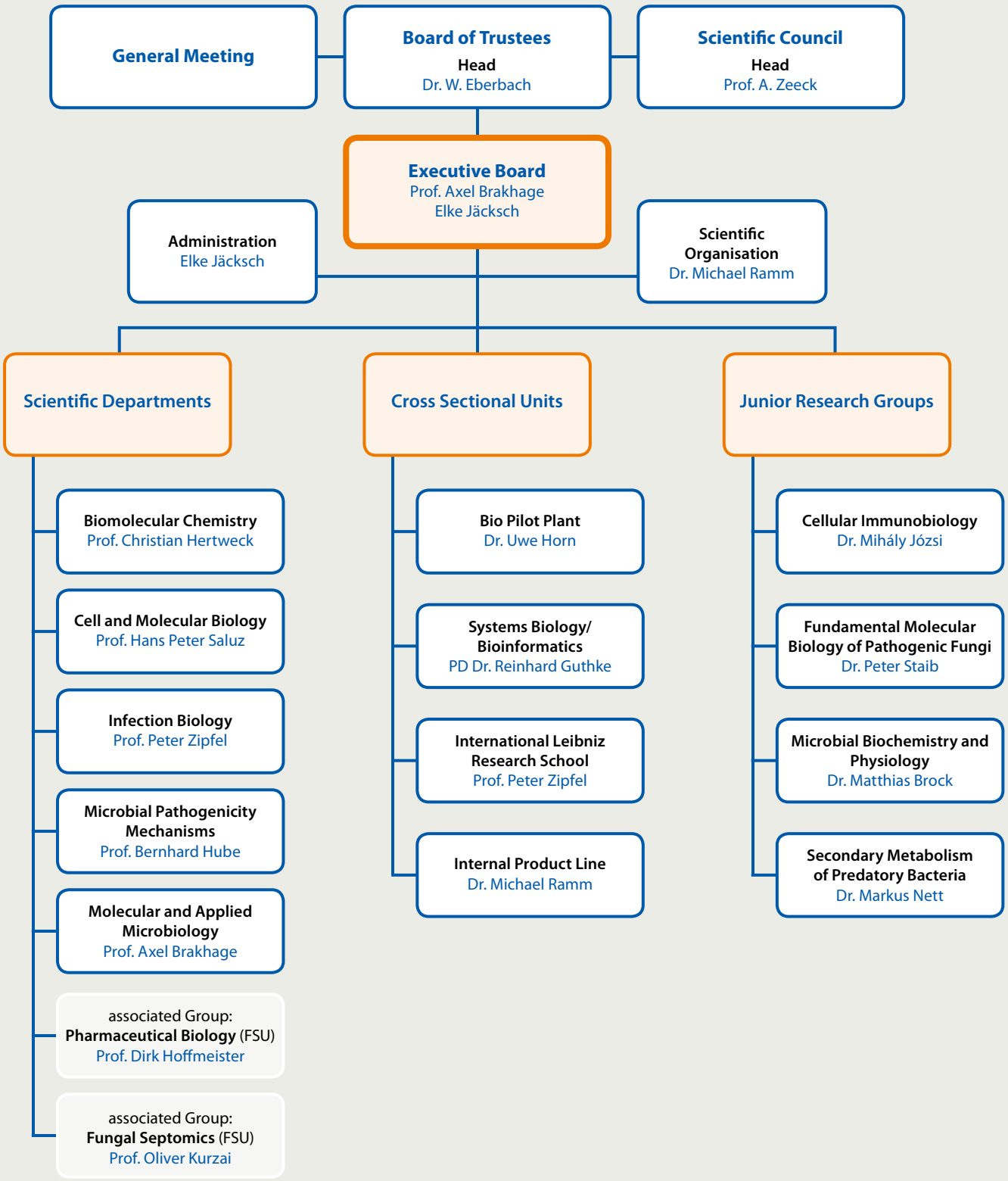
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Department of Biomolecular Chemistry

Department of Biomolecular Chemistry

The Department of Biomolecular Chemistry, headed by Prof. Christian Hertweck, focuses on various aspects of microbial secondary metabolism. The group covers the chemical and biochemical expertise in modern natural product research (structure elucidation, biosynthesis, enzymology, synthesis, interactions through biomolecules). In addition to the basic research projects, the department operates the institute's state-of-the-art analytical facilities (NMR, MS-techniques, MALDI-TOF) and supports other HKI departments and FSU groups with metabolite and proteome analyses.

The department also harbors the natural product discovery group, which is specialized in the isolation and structural elucidation of natural products by bioactivity-guided screening. This work is funded by DBU and BMBF and supported by various collaborations with industrial partners. Furthermore, the research group maintains the institute's central compound library. For the discovery of new potential therapeutics we investigate primarily novel types of microorganisms (endophytes, symbionts, little explored microorganisms) and apply genome-mining strategies (activation of silent gene clusters).

A major research area of the department is the investigation of biosynthetic pathways by a combination of chemical and biological me-

INTRODUCTION | EINLEITUNG

Head
Prof. Dr. Christian Hertweck

Die Abteilung Biomolekulare Chemie befasst sich unter der Leitung von Prof. Christian Hertweck mit der Chemie und Biologie des facettenreichen Sekundärmetabolismus von Mikroorganismen - Bakterien und Pilzen. In der Abteilung sind die chemischen und biochemischen Arbeitsgebiete der modernen Naturstoff-Forschung integriert (Strukturaufklärung, Biosynthese, Enzymmechanismen, Synthese und Naturstoff-basierte Interaktionen). Die für das Institut essentielle Hochleistungsanalytik (NMR, MS, MALDI-TOF) wird in der Abteilung Biomolekulare Chemie betrieben. Sie verfügt über ein breites Methodenspektrum zur Strukturaufklärung von niedermolekularen Verbindungen und zur Proteomanalyse, durch die insbesondere die HKI-Abteilungen und FSU-Arbeitsgruppen unterstützt werden.

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

Zur Suche nach neuen Naturstoffen werden vorwiegend neue Organismentypen (Endophythen, Symbionten, wenig untersuchte Mikroorganismen) verwendet und Genome mining-Strategien eingesetzt. Ein Schwerpunkt der Abteilung Biomolekulare Chemie liegt in der Aufklärung von mikrobiellen Biosyn-

thods. In nature, structural and functional diversity is governed by multienzyme processing lines that lead to highly complex compounds. To gain insights into the mechanisms and the evolution of the synthetic machineries the group investigates the functions of biosynthesis gene clusters and mechanisms of selected intriguing enzymes. For this purpose, we also do structural studies in collaboration. The majority of our projects is conducted in national and international scientific networks. In addition to understanding natural evolution of metabolic diversity we aim at harnessing the biosynthetic potential to produce novel natural product derivatives. In particular, biosynthetic pathways of pharmacologically relevant polyketides are investigated. Modern organic synthetic methods complement the microbes' biosynthetic capabilities.

Natural products represent mediators of biological communication, and their specific functions have developed during evolution. They play a key role in the interaction of microorganisms, in symbiosis and also in pathogenesis, e.g. in fungal infections. We have discovered the first cases, in which it is not the fungus that produces a mycotoxin, but bacterial symbionts that live within the fungal mycelium. This unique alliance of bacteria (*Burkholderia* sp.) and fungi (*Rhizopus microsporus*) produces the antimitotic polyketide rhizoxin, the

thesewegen über biologische und chemische Methoden. Die strukturelle und funktionelle Diversität entsteht in der Natur über Multi-Enzym-Prozesslinien, die zu hochkomplexen Verbindungen führen. Um die Mechanismen und die Evolution der Synthesemaschinerien biologischer Systeme zu verstehen, studiert die Arbeitsgruppe die Biosynthese-Gencluster und ausgewählte Enzyme. In Kollaboration werden hierzu auch strukturbiologische Projekte durchgeführt. Die meisten dieser interdisziplinären Arbeiten werden in überregionalen Netzwerken bearbeitet. Die Projekte haben nicht nur zum Ziel, ein besseres Verständnis von der Evolution metabolischer Diversität zu bekommen, sondern auch, das natürliche Biosynthesepotential zu nutzen, um neue Naturstoff-Derivate darzustellen. Bei den hierzu angewandten biokombinatorischen Techniken stehen Biosynthesewege pharmakologisch relevanter Polyketide im Mittelpunkt. Moderne Synthesemethoden und Biotransformationen komplementieren die Biosyntheseleistung der Mikroorganismen.

Naturstoffe sind Mediatoren der biologischen Kommunikation, die im Laufe der Evolution auf biologische Aktivität selektioniert worden sind. Sie spielen in der Interaktion von Mikroorganismen, bei Symbiosen und auch als Virulenzdeterminanten, zum Beispiel bei Pilzinfektionen eine wichtige Rolle. Wir haben die ersten beiden Fälle beschrieben, in denen nicht der Pilz ein Mykotoxin bildet, sondern bakterielle Symbionten, die im Pilzmyzel leben. Die einzigartige Allianz aus Bakterien

causative agent of rice seedling blight. In the department various aspects of this tripartite microbial interaction are being investigated, such as evolution of symbiosis and molecular basis of toxin biosynthesis. The biomolecular interactions in fungal-bacterial associations are jointly investigated with research groups at the HKI, the FSU, and the Max-Planck-Institute for Chemical Ecology in the context of the graduate schools ILRS and JSMC.

(*Burkholderia* sp.) und Pilzen (*Rhizopus microsporus*) bildet das antimitotisch wirksame Rhizoxin, das eine Schlüsselrolle bei der Reiskeimlingsfäule spielt. In der Abteilung Biomolekulare Chemie werden zahlreiche Aspekte dieser mikrobiellen Interaktion studiert, wie die Evolution der Symbiose und die molekulare Grundlage der Toxin-Biosynthese. Die biomolekularen Interaktionen in Pilz-Bakterien-Symbiosen sind Themen stark vernetzter Gemeinschaftprojekte der Arbeitsgruppen am HKI, der FSU und des Max-Planck-Instituts für chemische Ökologie im Rahmen der Graduiertenschulen ILRS und JSMC.

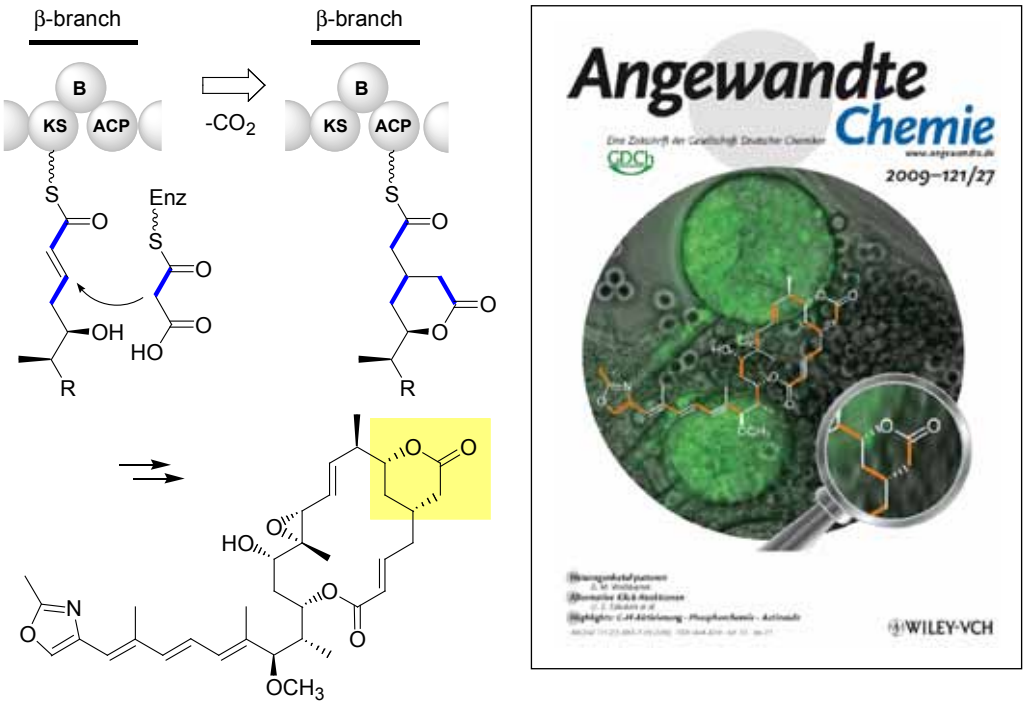


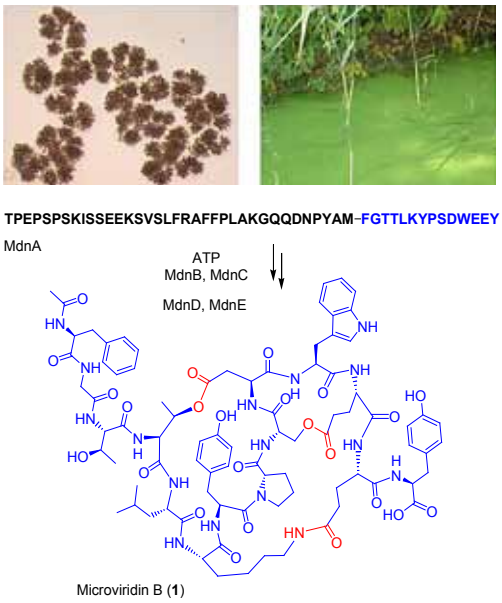
Figure 1-1
Model for polyketide chain branching in the biosynthetic pathway of the antimitotic agent rhizoxin; Angewandte Chemie cover art.

1 Biosynthetic Mechanisms and Processing Lines

Group Leader: Christian Hertweck

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

We have successfully engineered the rhizoxin biosynthetic assembly line to gain insight into the timing of polyketide chain branching and the module involved. The successful isolation and full structure elucidation of prematurely released pathway intermediates enabled the reconstruction of the final steps in polyketide assembly. Accordingly, the δ-lactone branch is introduced by a conjugate addition of a malonyl unit to an acryloyl precursor. Further mutational experiments provided strong evidence that the "branching module", consisting of KS, B ("branching"), and ACP domains, downstream of module 10 is involved in this non-canonical enzymatic C-C coupling. Polyketide synthases generally catalyze Claisen condensations for chain elongations, and to date, only three ways are known how nature introduces carbon branches into polyketides: by incorporating branched building blocks, by SAM-dependent methylation or by an isoprenoid-like mechanism. The Michael addition type β-branching mechanism in rhizoxin biosyn-



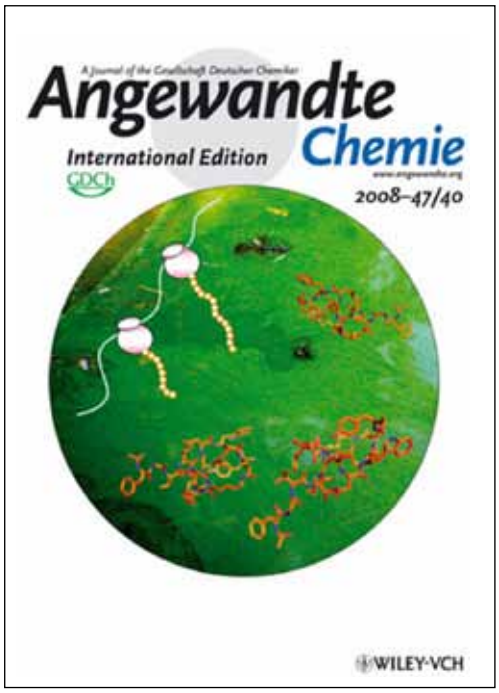


Figure 1-2
Ribosomal biosynthesis of tricyclic depsipeptides in cyanobacteria; Angewandte Chemie International Edition cover art.

thesis as proposed here is fully unprecedented and a novelty in the field of polyketides.

Various types of planctonic cyanobacteria such as *Microcystis* frequently form toxic blooms in freshwater lakes, leading to a dramatic decrease of biodiversity in these ecosystems. Some of the most remarkable cyanobacterial toxins belong to the microviridin family of tricyclic depsipeptides. All microviridins feature an unparalleled cage-like architecture and specifically inhibit various proteases. Microviridin J affects the molting process of *Daphnia* and is discussed as a feeding deterrent that may partly explain the enormous success of *Microcystis* species in the field. On the other hand, microviridin B has high therapeutic potential as an elastase inhibitor in the treatment of lung emphysema. Thus knowledge of the molecular basis of microviridin biosynthesis would not only allow monitoring of environment-threatening cyanobacterial blooms but also open the door to engineering valuable new therapeutics. In collaboration with Prof. Elke Dittmann, HU Berlin, we found that microviridins are synthesized from a ribosomal precursor peptide in *Microcystis* by a unique pathway involving stand-alone ATP grasp type ligases for ω-ester and ω-amide bond formation, as well as a specialized transporter-peptidase. The successful heterologous production of these unusual tricyclic depsipeptides in *E. coli* demonstrates for the first time that microviridins derive from a ribosomal peptide precursor. Reconstitution of the pathway *in vivo* and identification of microviridin variants shows that the leader peptide is processed by a transporter-peptidase and cyclized by two cyclases related to ATP grasp ligases. The utilization of these novel biocatalysts for depsipeptide formation from ribosomally produced peptides represents a new addition to the various known natural strategies for cyclopeptide biosynthesis and sets the ground for engineering microviridin variants.

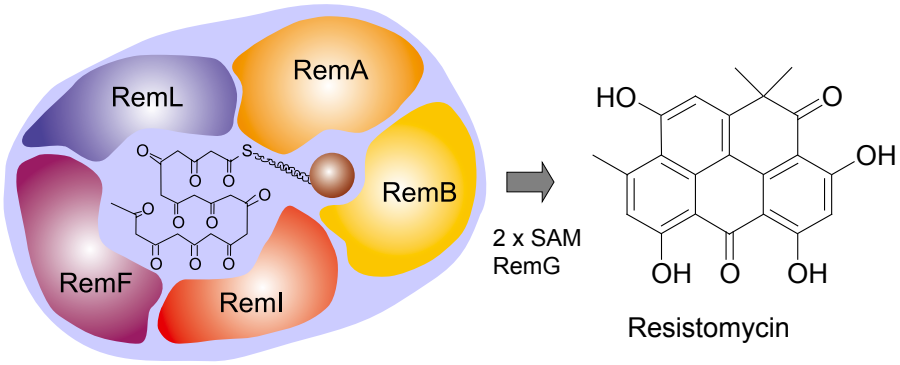


Figure 2-1
Formation of the antibiotic resistomycin by a multienzyme complex.

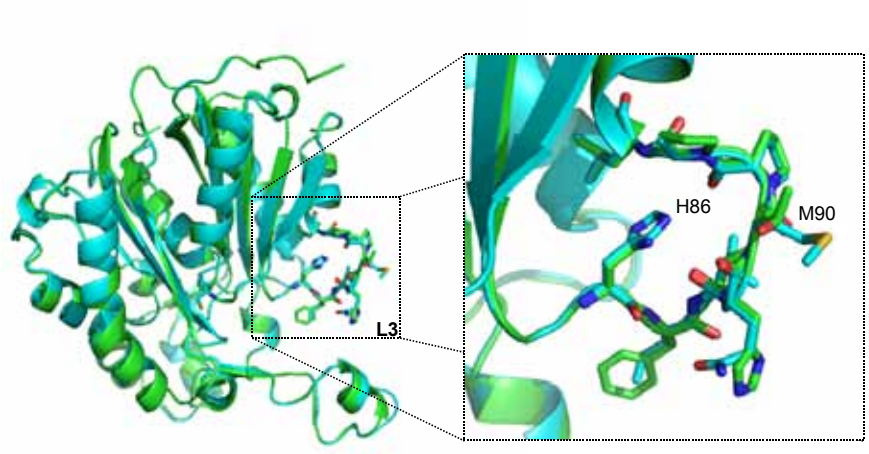


Figure 2-2
Superposition of the amino acid sequence of BenQ (green) onto the structure of ZhuH (cyan) highlighting the putative binding pockets at loop 3 (L3). The protrudent amino acid residues His86 and Met90 (from its counterpart of the dimer) of BenQ may be determinants for substrate specificity.

2 Engineered Biosynthesis of Aromatic Polyketides

Group Leaders: Zhongli Xu,
Christian Hertweck

Bacterial aromatic polyketides represent a large group of structurally diverse natural products many of which are medicinally relevant (e.g. tetracyclines, anthracyclines). With the advent of molecular tools and recombinant methods applicable to actinomycetes, it has become feasible to investigate bacterial aromatic polyketide biosynthesis at the genetic and biochemical levels, which has finally set the basis for engineering novel natural product derivatives. We are exploring the biosynthetic potential by rational mutagenesis and gene swapping and have applied this to various antibiotic biosynthetic pathways leading to polyphenols such as resistomycin, chartreusin and benastatin.

Resistomycin is a bacterial polyphenolic metabolite from *Streptomyces resistomycificus* with a unique pentacyclic 'discoid' ring system that clearly differs from the typical linear or angular architectures of aromatic polyketides. The first comprehensive cyclase amino acid sequence – function correlation revealed that the enzymes directing the nascent polyketide chain into a peri-fused system clearly differ from canonical linear and angular cyclases. All genes that are required and sufficient for resistomycin (*rem*) biosynthesis were identified through systematic dissection and reconstitution of the type II polyketide synthase (PKS) complex. The minimal rem PKS and the first cyclase were successfully cross-complemented with orthologs from the linear tetracenomycin polyketide pathway, indicating that both dekaketide pathways share early biosynthetic steps. In total three cyclases that are involved in discoid cyclization (RemI, RemF, and RemL) were identified by mutational analyses and *in vivo* pathway reconstitution.

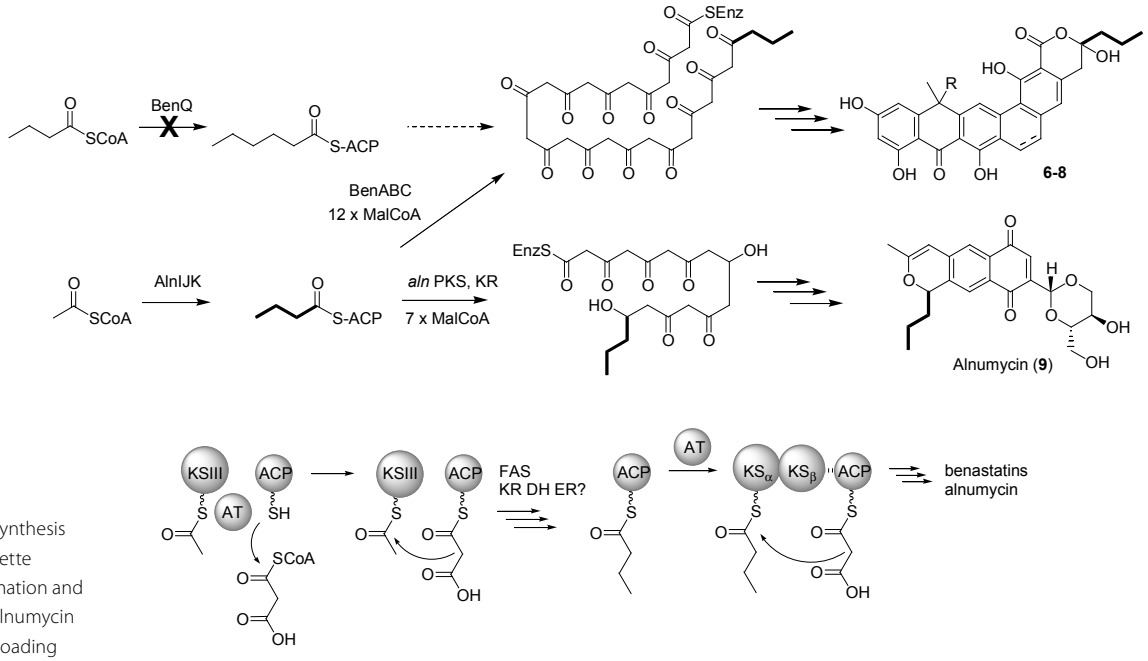


Figure 2-3
Models for alnumycin biosynthesis involving the priming cassette AlnIJK for butyryl-ACP formation and engineered a benastatin-alnumycin hybrid pathway by KAS III loading domain swapping.

Analyses of the metabolic profiles of mutants expressing incomplete gene sets led to the discovery of a novel tetracenomycin derivative, TcmR1. The most surprising finding is that only the concerted action of the PKS and all three cyclases leads to the discoid ring structure. These results provide strong support for a model according to which the multienzyme complex forms a cage in which the polyketide is shaped, rather than a sequential cyclization of the polyketide chain by individual enzymes.

Benastatins are aromatic polyketides from *Streptomyces* spp. that efficiently inhibit glutathione-S-transferases and induce apoptosis. Their biosynthesis involves a type II polyketide synthase, and a keto acyl synthase (KAS) III component (BenQ) similar to FabH that is crucial for providing and selecting the rare hexanoate PKS starter unit. The function of BenQ as a KAS III was experimentally proven by point mutation of the active site cysteine. In the mutant several novel short chain fatty acid derived penta- and hexacyclic benastatin derivatives with antiprolieferative activities are formed. Strategies for engineering benastatin biosynthesis were attempted. Synthetic starter unit surrogates were not incorporated by block mutants, suggesting that the primer needs to be enzyme-bound. Thus, on the basis of KAS III crystal structures the three-dimensional structure of BenQ was modeled and the predicted substrate binding tunnel was altered by individual mutations of potential gatekeeping residues (H95A and M99A). However, no significant changes in substrate specificity were observed, indicating that there are other or additional gatekeeping amino acid residues in BenQ or secondary factors including likely protein – protein interactions between BenQ and the PKS complex, and possible conformational changes in BenQ. Finally, a benQ null mutant was complemented with butyrate starter unit biosynthesis genes from the alnumycin biosynthesis gene cluster, which resulted in a great (10×) enhancement

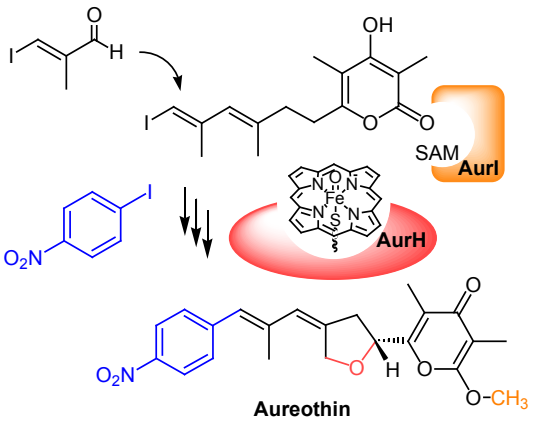


Figure 3-1
The first asymmetric synthesis of the antiproliferative and antifungal pyrone metabolite (+)-*R*-aureothin from *Streptomyces thioluteus* was completed by taking advantage of synergizing synthetic and enzymatic transformations.

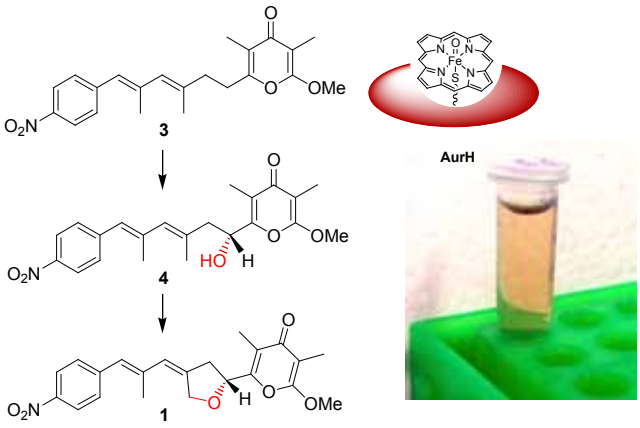


Figure 3-2
In vitro biotransformations using a cytochrome P450 monooxygenase AurH from the aureothin pathway provide the first direct experimental evidence that a single monooxygenase is capable of sequentially installing two C-O-bonds forming a tetrahydrofuran ring.

in the production of butyrate-primed hexacyclic benastatin derivatives. The successful generation of a alnumycin-benastatin FAS-PKS hybrid pathway highlights the potential of metabolic pathways, which may lead to novel potential therapeutics and increased yields of desired natural products.

3 Chemobiosynthesis and Biocatalysis

Group Leaders: Robert Winkler, Susanne Schneider, Christian Hertweck

The synthesis of therapeutics by taking advantage of the synergism of synthetic and enzymatic transformations is an emerging field. Recently, we have accomplished the first asymmetric synthesis of the antiproliferative agent (+)-*R*-aureothin by a chemoenzymatic approach. Aureothin is a densely functionalized polyketide metabolite of *Streptomyces thioluteus* that efficiently stalls proliferation of va-rious tumor cells. A number of related bacterial natural products share the unusual nitroaryl and tetrahydrofurylpyrone moieties, such as the HIV protease inhibitor neoaureothin (spectinabilin), and the immunosuppressant SNF 4435C/D. Notably, the preparation of the chiral furyl segment proved to be most challenging due to facile racemization and as of yet no enantioselective total synthesis of (+)-*R*-aureothin has been reported. In our chemoenzymatic approach, the polyketide backbone was assembled in a modular fashion by chemical synthesis and tailored enzymatically by the regioselective pyrone methyl transferase AurI and the bifunctional cytochrome P450 monooxygenase AurH that is capable of installing two C-O bridges. Besides deoxyaureothin we succeeded in introducing the oxa heterocycle into a bioisosteric vinyl iodide by asymmetric enzymatic oxygenation. The resulting synthetic building block was successfully employed for a Stille coupling yielding (+)-*R*-aureothin, as confirmed by CD spectroscopy. Although

cytochrome P450 enzymes have been used extensively in biotransformations, to the best of our knowledge these results represent the first example for an asymmetric cytochrome P450–mediated furan heterocyclization used in synthesis. Our modular approach will set the basis for the synthesis of various analogues of the aureothin family of polyketides.

Biosynthetic pathways leading to complex secondary metabolites frequently involve enzymes with intriguing synthetic capabilities. A closer investigation of such enzymes may lead to novel biocatalysts that could complement the currently available synthetic repertoire. Complex polyketide metabolites like macrolides, polyenes, and polyethers are an important source of novel therapeutics or an inspiration for their development. Many of these compounds feature medium-sized heterocycles that lend rigidity to the carbon backbone and thus support a defined spatial arrangement of the substituents. Various biosynthetic routes to O-heterocycles have been reported. However, the tetrahydrofuran ring of aureothin is biosynthesized in a completely different manner. Through biochemical and chemical analyses we could provide direct experimental evidence that the sequential heterocycle formation is catalyzed by a single enzyme (AurH) both in *in vivo* and *in vitro* and reveal the stereochemical course and order of this unprecedented oxygenation reaction. We have overproduced and purified the unusual cytochrome P450 monooxygenase AurH *in E. coli* and reconstituted its activity *in vitro*. For the first time we have demonstrated by successful in vitro biotransformations that a single cytochrome P450 monooxygenase is capable of sequentially installing two C-O bonds. In trapping a chiral hydroxyl intermediate and elucidation of its absolute configuration we have established the order of the oxygenation reactions as well as the stereochemical course of the reaction. The unusual heterocyclization can be rationalized on the basis of current cytochrome P450 enzyme models. Even so, AurH is a novel biocatalyst that promotes a biotransformation that is as yet impossible to emulate by synthetic methods or other known enzymes.

4 Genome Mining for Natural Product Discovery

Group Leaders: Kirstin Scherlach, Keishi Ishida, Christian Hertweck

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

We found an unassigned PKS cluster in the genome of *Burkholderia thailandensis*, from which no polyketide has been reported to date. In collaboration with Prof. J. Piel, University of Bonn, we have analyzed the orphan locus, which encodes 16 PKS modules and one non-ribosomal peptide synthetase (NRPS) module. Since KS analysis predicted an extended conjugated double bond system, we monitored the metabolic profile of *B. thailandensis* by HPLC-DAD-MS for nitrogen-containing polyenes. This revealed the transient occurrence of two compounds with a characteristic polyene absorption exclusively in the early growth phase. The isolation from an upscaled culture was challenging due to rapid temperature- and light-induced decomposition. Careful workup provided a pure sample of thailandamide A, and NMR analysis allowed its full structure elucidation. Thailandamide A exhibits novel structures with an unprecedented 4-hydroxyphenylacetate starter unit, and represents a rare example of a polyketide from bacteria of the genus *Burkholderia*.

Fungi produce numerous low-molecular weight molecules endowed with a multitude of biological activities. However, mining the full genome sequences of fungi indicates that their potential to produce secondary metabolites is greatly underestimated. Since most of the biosynthesis gene clusters are silent under laboratory conditions, one of the major challenges is to understand the physiological

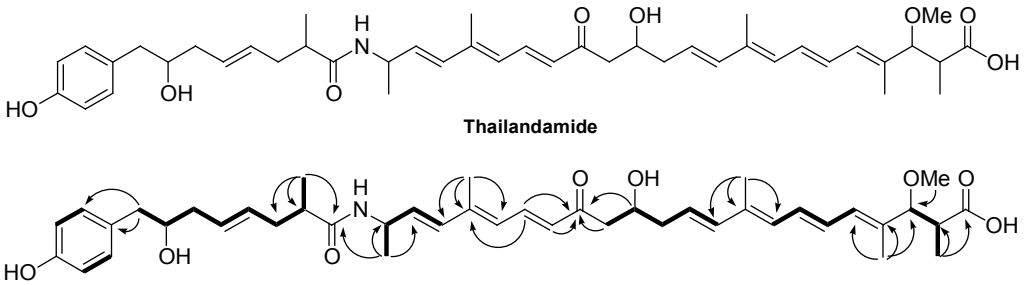
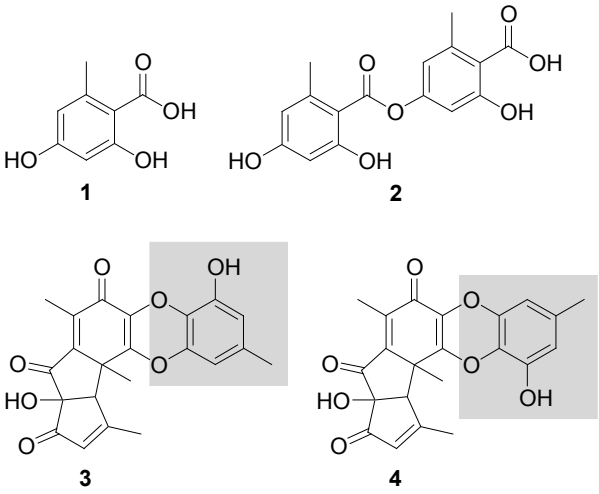


Figure 4-1
Structure of thailandamide A and key 1H-1H COSY (bold lines) and HMBC (arrows) correlations.



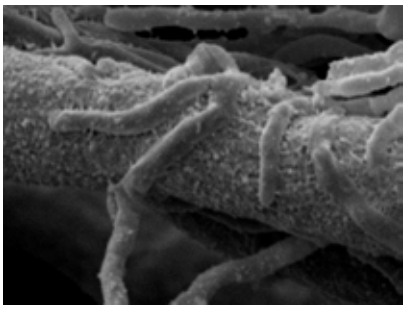
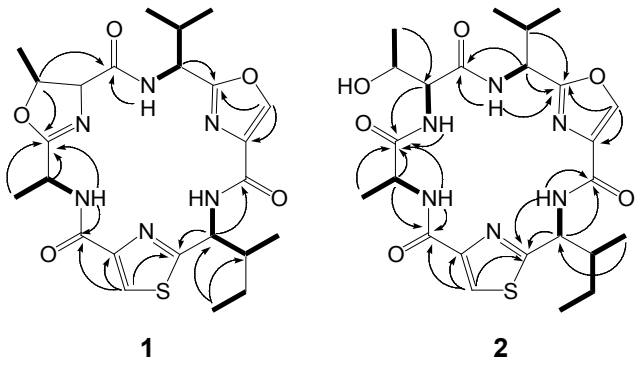


Figure 4-2
Induction of fungal metabolite production through bacterial interaction.

conditions under which these genes are activated. In collaboration with the Department of Molecular and Applied Microbiology, Prof. A. Brakhage, we searched for ways to bring cryptic fungal metabolited to light. For this purpose, the important model fungus *Aspergillus nidulans* was cocultivated with a collection of 58 soil-dwelling actinomycetes. By microarray analyses of both *Aspergillus* secondary metabolism and full genome arrays, Northern blot and qRT-PCR analyses we demonstrate for the first time at the molecular level that a distinct fungal-bacterial interaction leads to the specific activation of fungal secondary metabolism genes. Most surprisingly, dialysis experiments and electron microscopy indicated that an intimate physical interaction of the bacterial and fungal mycelia is required to elicit the specific response. Gene knock out experiments provided evidence that one induced gene cluster codes for the long sought-after polyketide synthase required for the biosynthesis of the archetypal polyketide orsellinic acid, the typical lichen metabolite lecanoric acid, and the cathepsin K inhibitors F-9775A and F-9775B. A phylogenetic analysis demonstrates that orthologs of this polyketide synthase are widespread in nature, in all major fungal groups including mycobionts of lichens. These results provide evidence of specific interaction between microorganisms belonging to different domains and support the hypothesis that not only diffusible signals, but also intimate physical interactions contribute to the communication between microorganisms and induction of otherwise silent biosynthesis genes.

Figure 4-3
The key COSY (bold lines) and HMBC (arrows) correlations of microcyclamide 7806A (**1**) and 7806B (**2**).



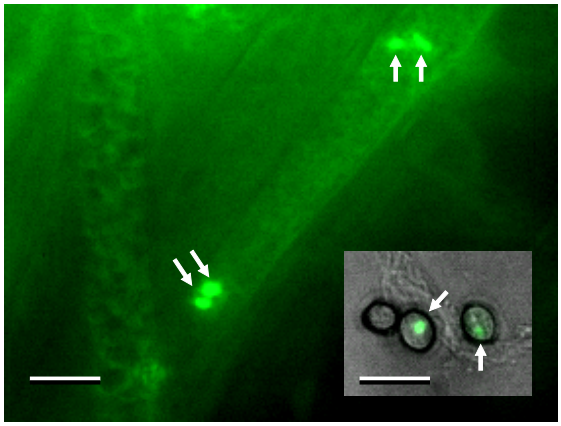


Figure 5-1
Confocal laser scanning micrographs of rhizoxin-producing bacterial endosymbionts (*Burkholderia rhizoxinica*, labeled with GFP) in the mycelium of *Rhizopus microsporus* (main image) and in spores (inset). Bars: 10 mm (main image), 5 mm (inset).

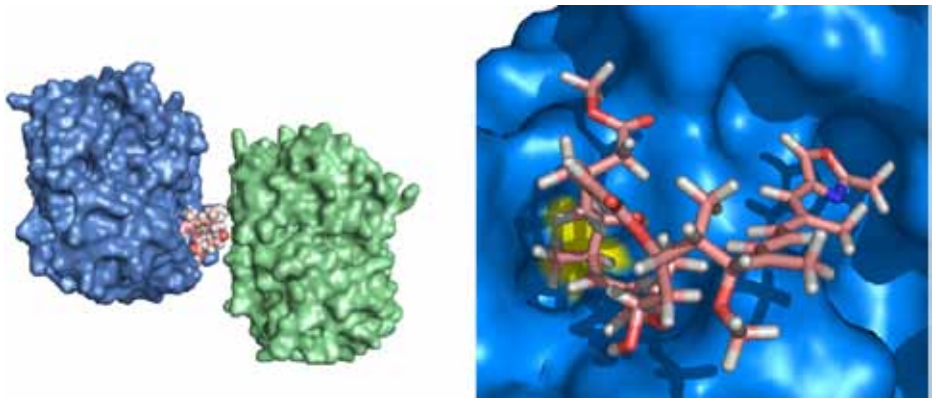


Figure 5-2
Modeled rhizoxin binding to β-tubulin at the interface of the α,β-heterodimer. Position Asn100 is marked in yellow.

In collaboration with Prof. E. Dittmann, HU Berlin, we were able to apply a genome mining approach to detect novel members of the microviridin family of cyanobacterial metabolites. In the freshwater cyanobacterium *Microcystis aeruginosa* NIES 298 the cytotoxic peptide is formed by a ribosomal pathway. Genome mining of a *Microcystis aeruginosa* PCC 7806 guided the discovery and structural elucidation of two novel cyclopeptides, microcyclamide 7806A and 7806B.

5 Microbial Interactions: Investigations of Endofungal Bacteria

Group Leaders: Laila P. Partida Martinez, Christian Hertweck

Mycotoxins are compounds of fungal origin that can adversely affect human, animal and plant health through food spoilage or infection, even to the point of epidemics such as turkey X disease and ergotism. The biosynthetic pathways of various mycotoxins (such as aflatoxin and fumonisins) are generally well understood. However, we have discovered two examples where a mycotoxin is not synthesized by the fungus itself but by bacteria residing within the fungal cytosol. These discoveries have implications in various fields, in particular ecology, medicine and food processing.

The rice seedling blight fungus *Rhizopus microsporus* harbors endosymbiotic *Burkholderia* spp. for the production of the virulence factor, the antimitotic agent rhizoxin. Since the toxin highly efficiently blocks mitosis in most eukaryotes, it remained elusive how self-resistance emerged in the fungal host. We have systematically correlated rhizoxin sensitivity with the nature of β-tubulin sequences in the kingdom fungi. 49 new β-tubulin sequences were generated for representative species of Ascomycota, Basidiomycota and Zygomycota. Rhizoxin sensitivity assays revealed two fur-

ther amino acids at position 100 (Ser-100 and Ala-100), in addition to the known Ile-100 and Val-100, which convey rhizoxin resistance. All sensitive strains feature Asn-100. This hotspot was verified by modeling studies, which support the finding that rhizoxin preferentially interacts with the tubulin molecule in a cavity near position 100. Ancestral character state reconstructions conducted in a Bayesian framework suggest that rhizoxin sensitivity represents the ancestral character state in fungi, and that evolution of rhizoxin resistance took place in the ancestor of extant resistant Zygomycota. These findings support a model according to which endosymbiosis became possible through a parasitism – mutualism shift in insensitive fungi.

To investigate the global distribution of the toxinogenic fungal-bacterial symbiosis, we studied eight bacterial endosymbiont strains isolated from toxinogenic *Rhizopus microsporus* strains in pure culture. All isolates are representatives of the same unique "endofungal" ecotype, albeit the hosts' origins cover all five continents and occur in highly diverse niches. The bacterial endosymbionts share characteristic phenotypic traits like secondary metabolite production and protein profile, as demonstrated by HPLC-MS and MALDI-TOF, respectively. Phylogenetic analyses (16S rDNA) provide strong evidence that all symbiont strains originate from a common ancestor and form a new complex within the genus *Burkholderia*. This observation is strongly supported by multilocus sequence typing, according to which all eight symbiont isolates can be grouped into continental branches. Results revealing both similar and deviating geographical grouping of fungal isolates in comparison to bacterial endosymbionts allow hypothesizing about possible cospeciation of fungal and bacterial symbionts and some extent of horizontal transmission events. All bacterial strains investigated seem to have evolved mainly separately from each other, not showing extensive recombination. In addition, we present preliminary evidence that there might be a mutational bias towards higher AT-contents, as it is known from other endosymbiotic bacteria.

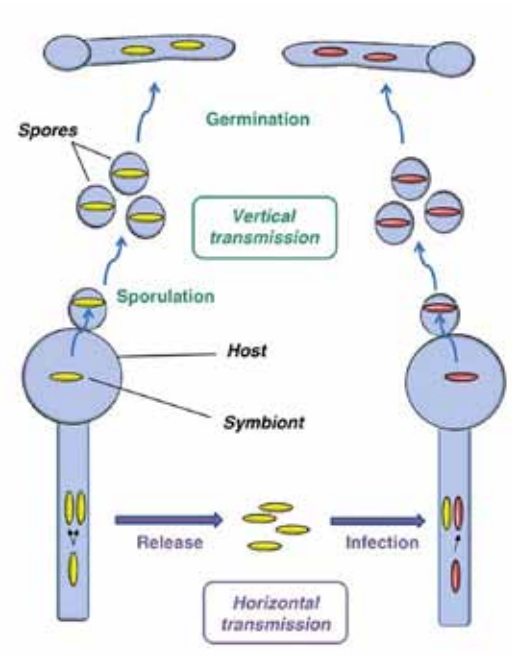


Figure 5-3
Hypothetical lifecycle of endofungal bacteria and their hosts. Endobacteria replicate within the fungal mycelium. During vertical transmission they enter vegetative spores and are transferred to the next generation. Horizontal transmission involves release from the host cell and infection of another compatible host organism.

6 Mass Spectrometry Based Protein and Peptide Analysis

Group Leader: Robert Winkler

The department of Biomolecular Chemistry operates the institute's peptide and protein analysis platform. The department is equipped with a MALDI-TOF/TOF, an ESI-QTOF and a nanoLC-ESi-ion trap with ETD option (Figure 6-1). Up to 200 proteomics samples can be measured per day on the MALDI-TOF/TOF. The analysis of the data is performed on in-house servers, which also allows the usage of unpublished genomic databases. We have established a set-up that allows the measurement of proteomic samples with extremely low peptide concentrations or peptide mixtures by nanoLC-MS.

This technique has been applied in various in house and external collaborations (FSU). Important examples include the generation of a

Figure 6-1
Mass spectrometers used for protein and peptide analysis: ESI-QTOF, nanoLC-MS and MALDI-TOF/TOF

two-dimensional proteome reference map for the human pathogenic filamentous fungus *Aspergillus fumigatus*, as well as the identification of pyomelanin in this organisms. Protein MS also supported the discovery of a novel immune evasion strategy of *Candida albicans* involving proteolytic cleavage of a salivary antimicrobial peptide. Furthermore, we assisted in the identification of thioredoxin and epidermal-fatty acid binding protein as up-regulated protein markers in microdissected tumor tissue. In addition, we have studied protein and peptide modifications, such as protease degradation of peptide antibiotics, phosphorylation/dephosphorylation, cofactors of proteins, as well as artificial modification of proteins. For quick identification of microorganisms we now also use a modern MALDI-TOF based approach (biotyping).

7 Screening for Bioactive Natural Products

Group Leaders: Isabel Sattler, Ling Ding, Christian Hertweck

The research group "Natural Products Discovery" focuses on the exploitation of structural diversity from a broad spectrum of natural sources for providing new compounds for understanding biosynthetic capacities of the producing organisms and for biological characterization, e.g. in drug discovery. We are relying on a number of different resources for bioprospecting. In the last two years, our major natural products screening programs involve extremophilic and rare actinomycetes, as well as filamentous fungi, from unusual habitats from the southwest of China (provided by Key Laboratory for Microbial Resources, Yunnan University, Kunming, P.R. China), endophytes from mangrove plants from southeast China (plant material provided by National Research

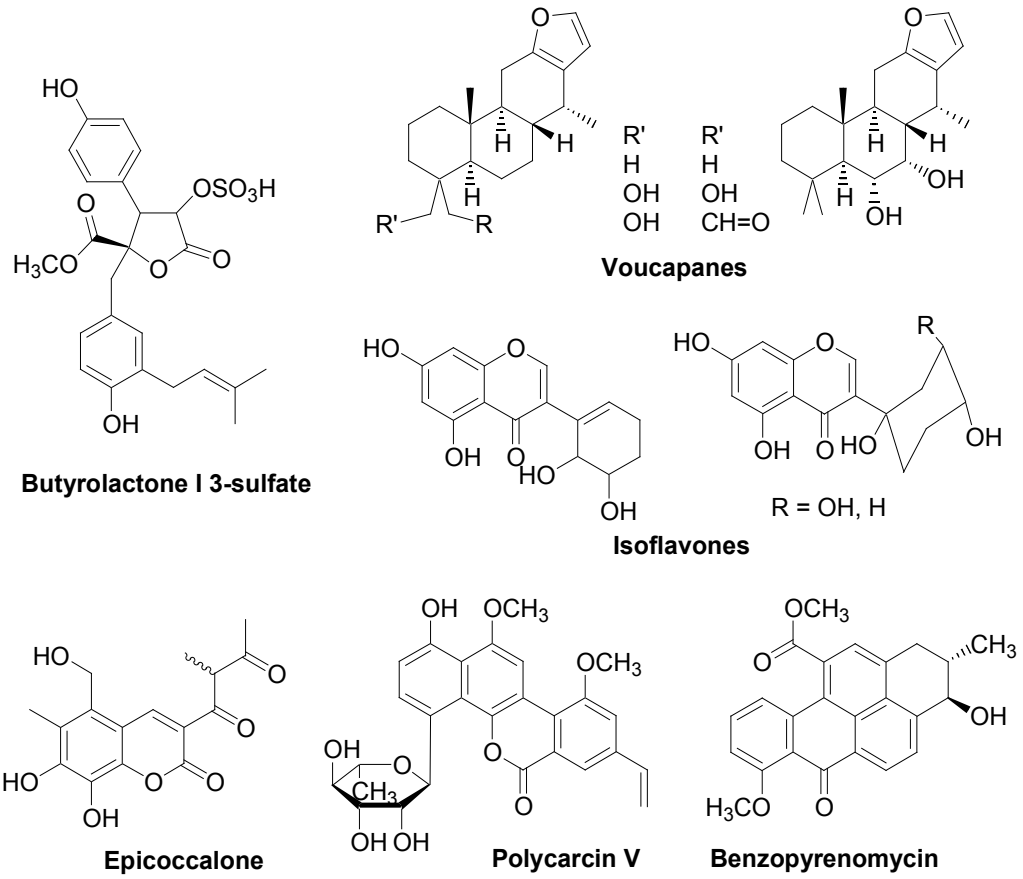


Figure 7-1
Structures of selected biologically active compounds published in 2008-2009

Laboratories of Natural and Biomimetic Drugs, Peking University Beijing, P.R. China), and rare actinomyces from heavy-metal contaminated habitats, e.g. from the Feengrotten, a former alum slate mine near Saalfeld (Thuringia) and former uranium mining area around Ronneburg, Thuringia (together with Institute of Microbiology, Friedrich Schiller University Jena).

In the search for new natural products, we efficiently intertwine biologically driven approaches, e.g. antimicrobial and cytotoxicity screening, with the physico-chemical analysis of the complex compound mixtures extracted from the producing organisms. Combined with the particular scientific objectives of our projects, we continuously thrive to optimize our screening procedures in order to improve methods for the effective exploitation of natural products. A particular focus on advancing our screening routines is set on improving dereplication by HPLC/MS^n-analysis, including a database with HPLC/UV/MS data of over four thousand natural products, and HPLC/MS-driven separation on the analytical and preparative scale. For biological testing we rely on our colleagues of the IPL team and external collaboration partners. Our major goal is to access meaningful, and best, structurally new compounds in the subsequent chemical projects including chromatographic isolation and structure elucidation. In our screening program on endophytic microorganisms form mangrove trees, we identified several seco-oleane-type triterpenes from *Phomopsis* sp. isolated from *Hibiscus tiliaceus*. Epicoccalone, a coumarin-type chymotrypsin inhibitor was obtained from an *Epicoccum* sp. associated with a tree fungus. Analysis of the *Aspergillus terreus* metabolome revealed unusual butyrolactone I derivatives from carrying sulfate moieties.

Cytotoxic, anti-proliferative and antimicrobial furanoditerpenoids (voucapane, voucapane-

6α,7α-diol, voucapane-18,19-diol and 18-hydroxyvoucapan-19-al) were isolated from *Stuhlmania moavi*. Investigation of *Streptomyces polyformus* showed a surprising plasticity in gilvocarcin-type C-glycoside pathways. Cytotoxicity profiling of polycarcin V in a panel of 37 tumor cell lines indicated significant cytotoxic activity with a pronounced selectivity for non-small cell lung cancer, breast cancer and melanoma cells. Six novel isoflavone derivatives along with four known isoflavones were isolated from a culture of a highly nickel-resistant strain of *Streptomyces mirabilis* from a former uranium mining area. The unprecedented types of non-aromatic, hydroxylated B rings result from plant isoflavone biotransformation. All new compounds display weak cytotoxic but potent antiproliferative activities. The anti-oestrogenic properties of one congener against MCF-7 human breast cancer cell line (GI_{50}: 6 μM) is even higher than the reference compound genistein. Perhaps one of the most unusual of our recent discoveries is benzopyrenomycin from of *Streptomyces lavendulae*. Benzopyrenomycin represents the first natural product with a carbacyclic benzo[a] pyrene ring system. Four angucyclic congeners were identified in the broth of *S. lavendulae*, out of which rubiginone A2 shows an identical exocyclic substitution pattern. This finding provides strong evidence for a model according to which benzopyrenomycin is biosynthesized by condensation of an angucyclic anthrone precursor with a C3/C4 building block such as oxaloacetate. A biological evaluation of the novel compound revealed a significant activity against various tumor cell lines.

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Deutsche Forschungsgemeinschaft
Schwerpunktprogramm 1152: Evolution metabolischer Diversität
Nicht-colineare Thiotemplat-Systeme als Modell für die Evolution von Polyketid-synthasen
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Deutsche Forschungsgemeinschaft
Biosynthese von Nitroverbindungen: Struktur und Funktion einer Mangan-abhängigen N-Oxygenase
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Teilprojekt: Neue antibakterielle und antitumorale Polyketide durch Biokombinatorik
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Bundesministerium für Bildung und Forschung
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ProExzellenz Initiative
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Selected publications
(HKI authors in bold)

Nguyen T, **Ishida K**, Jenke-Kodama H, Dittmann E, Gurgui C, Hochmuth T, Taudien S, Platzer M, **Hertweck C**, Piel J (2008) Exploiting the mosaic structure of trans-acyl transferase polyketide synthases for natural product discovery and pathway dissection. *Nature Biotechnol* 26, 225-233.

Huang X, **He J**, Niu X, **Menzel KD**, **Dahse HM**, **Grabley S**, Fiedler P, **Sattler I**, **Hertweck C** (2008) Benzopyrenomycin, a cytotoxic bacterial polyketide metabolite with an unprecedented benzo[a]pyrene-type carbacyclic ring system. *Angew Chem Int Ed* 47, 3995-3998.

Ziemert N, **Ishida K**, Liaimer A, **Hertweck C**, Dittmann E (2008) Ribosomal synthesis of tricyclic depsipeptides in bloom-forming cyanobacteria. *Angew Chem Int Ed* 47, 7756-7759.

Fritzsche K, **Ishida K**, **Hertweck C** (2008) Orchestration of discoid polyketide cyclization in the resistomycin pathway. *J Am Chem Soc* 130, 8307-8316.

Richter MEA, **Traitcheva N**, **Knüpfer U**, **Hertweck C** (2008) Sequential asymmetric polyketide O-heterocyclization catalyzed by a single cytochrome P450 monooxygenase (AurH). *Angew Chem Int Ed* 47, 8872-8875.

Ziehl M, **Hertweck C** (2008) Chemoenzymatic total synthesis of the antiproliferative polyketide (+)-R-aureothin. *ChemBioChem* 9, 2064-2066.

Kusebauch B, **Busch B**, **Scherlach K**, **Roth M**, **Hertweck C** (2009) Polyketide chain branching by an enzymatic Michael addition. *Angew Chem Int Ed* 48, 5001-5004.

Schroeckh V, **Scherlach K**, **Nützmann HW**, **Shelest E**, **Schmidt-Heck W**, **Schuemann J**, **Martin K**, **Hertweck C**, **Brakhage AA** (2009) Intimate bacterial-fungal interaction triggers biosynthesis of archetypal polyketides in *Aspergillus nidulans*. *Proc Natl Acad Sci USA* 106, 14558-14563.

Lackner G, **Moebius N**, **Scherlach K**, **Partida-Martinez LP**, **Winkler R**, **Schmitt I**, **Hertweck C** (2009) Global distribution and evolution of a toxinogenic *Burkholderia rhizopus* symbiosis. *Appl Environ Microbiol* 75, 2982-2986.

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Department of Infection Biology

Department of Infection Biology

The department of Infection Biology characterizes the role of the complement system, an essential and central immune surveillance system for the elimination of infectious microbes and for the clearance of altered self cells. Human regulators restrict complement activation and are essential for maintaining tissue integrity and homeostasis. Human pathogenic fungi, like many pathogenic microbes evade host immune and complement attack by expressing surface proteins that bind human complement and innate immune regulators. The identification of such microbial surface proteins and the molecular characterization of these evasion strategies, reveals general immune escape mechanisms and identifies novel virulence factors that represent interesting vaccine candidates and new targets for immune intervention.

The same human immune regulators, that are utilized by pathogenic microbes for complement evasion are central players and effectors that maintain tissue integrity and homeostasis. The inhibitor Factor H protects human cells during immune stress, and consequently mutations and genomic deletions result in dysfunction that cause autoimmune diseases. We have identified important disease mechanisms and define how Factor H gene mutations and deletion of the *CFHR1/CFHR3* genes cause kidney and retinal disorders. Diseases that are as-

INTRODUCTION | EINLEITUNG

Head:
Prof. Dr. Peter F. Zipfel

Die Abteilung Infektionsbiologie beschäftigt sich mit der Rolle des Komplementsystems bei der Immunevasion von pathogenen Mikroorganismen und der Immundysfunktion bei Autoimmunerkrankungen. Pathogene Mikroorganismen exprimieren Oberflächenproteine, die als Rezeptoren für lösliche Immun- und Komplementregulatoren des Wirtes dienen, und die eine wichtige Funktion bei der Immunevasion spielen. Die Identifizierung und funktionelle Charakterisierung dieser mikrobiellen Proteine zeigt einen generellen Mechanismus der Immunevasion und führt zur Identifizierung von neuen Virulenzfaktoren, welche für das Überleben des Pathogens im Menschen als immunkompetentem Wirt entscheidend sind. Die von pathogenen Mikroorganismen genutzten Komplementregulatoren des Wirtes spielen eine zentrale Rolle bei der Aufrechterhaltung der Gewebsintegrität im menschlichen Organismus. Mutationen in diesen Regulatorproteinen führen zur Immundysfunktion und Autoimmunerkrankungen der Niere, wie Membranoproliferativer Glomerulonephritis, der atypischen Form des Hämolytisch Urämischen Syndroms und definierte Sequenzvariationen einzelner Gene erhöhen das Risiko für die altersabhängige Makuladegeneration des Auges (AMD). Dieser Ansatz erlaubte es eine neue autoimmune Form von HUS zu definieren, die als DEAP-HUS (defizient für CFHR-Proteine und positiv für Autoantikörper) bezeichnet wird. Die meist jugendlichen DEAP-HUS Patienten benötigen eine besondere Diagnose und spezielle Therapie. Bei frühzeitiger und geeigneter immunsuppressiver Behandlung können diese jugendlichen Patienten in der Regel vor Nierenversagen und einer langjährigen Dialysebehandlung bewahrt werden.

sociated with defective complement regulation include hemolytic uremic syndrome (HUS) and membranoproliferative glomerulonephritis (MPGN) and also the retinal disease age related macular degeneration (AMD) that represents a common form of blindness in the elderly population. This approach allowed to define a novel autoimmune form of HUS termed DEAP-HUS (Deficient for CFHR-proteins and autoantibody positive HUS). DEAP-HUS patients require diagnosis and therapy and when properly treated kidney defects and long term dialyses can be prevented in these mostly juvenile patients.

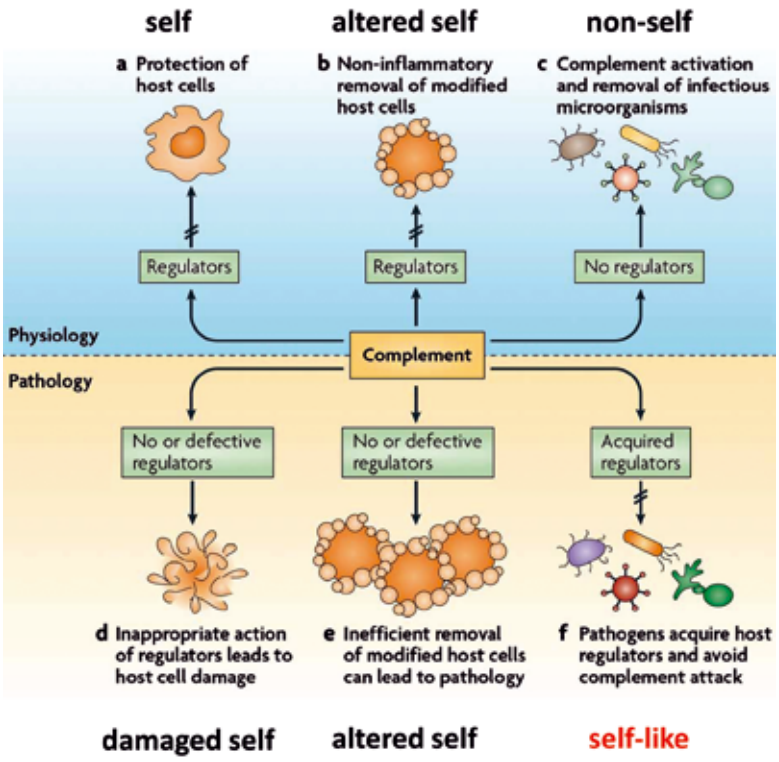


Figure 1
The benefits and risk of complement.
Complement is a central immune surveillance system that protects the surface of host cells and biomembranes, assists in the removal of altered self cells such as apoptotic cells and particles as well as necrotic debris and is relevant for the elimination of infectious microbes. Defects in this central pathway by gene mutations or by pathogenic surface structures results in damage of self and in disease.

1 Immune evasion of human pathogenic yeasts and fungi

Group Leader: Peter F. Zipfel

Human pathogenic yeasts use sophisticated strategies to evade host innate immune and complement attack. These mechanisms are both common and unique and the members of the Department of Infection Biology have identified novel immune evasion proteins that are used by different pathogenic microbes such as *Candida albicans* as well as by other pathogenic yeasts and fungi (Figure 1). In addition a second, novel and timely separated evasion mechanism for control of the host complement attack was identified which is shared by *Candida albicans, Arthroderma benhamiae* and *Aspergillus fumigatus*. The three pathogenic fungi secrete complement degrading proteases that degrade central components of the complement cascade and block host complement activation.

A novel immune evasion protein of the human pathogenic yeast *C. albicans* was identified. This CaCRASP2, that is also termed Pra1 (pH regulated antigen1) represents a fungal Factor H, FHL1 and plasminogen binding surface protein. CaCRASP2 is a 56 kDa glycosylated protein, composed of 299 amino acids that displays multiple functions and that has different sites of action. As a surface protein, expressed both by yeast and hyphae, *Candida* CRASP2 acquires several human plasma proteins and thus aids in complement and immune evasion. CaCRASP2 is also secreted by both yeast cells and hyphae and in the direct surroundings of the fungal cell secreted CaCRASP2 controls complement activation by enhancing Factor H mediated cofactor activity and by inhibiting C3 inactivation. In addition secreted CaCRASP2 also binds to the surface of human immune cells and specifically binds to the human integrin receptor CR3 (also termed CD11b/CD18 or $\alpha_M\beta_{II}$), that is expressed on the surface of human maro-

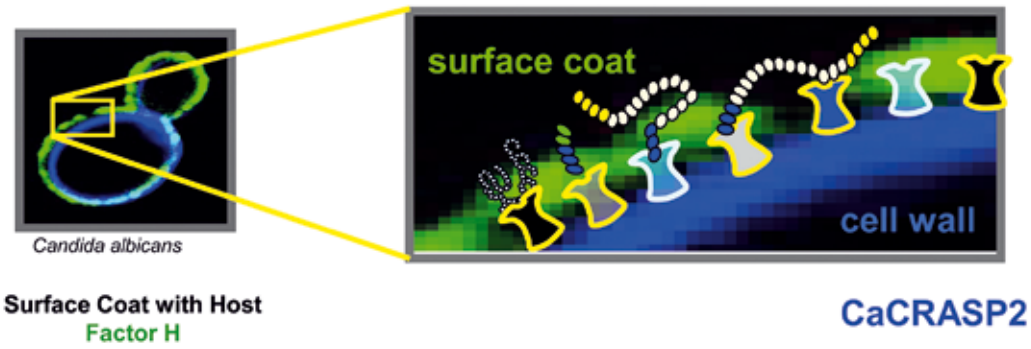


Figure 2
The human pathogenic yeast *Candida albicans* uses specific surface proteins to acquire human regulators and effector proteins.
Candida expresses several surface proteins that are termed CRASP1 – CRASP5 (Complement Regulator Acquiring Surface Proteins) that bind human complement regulators Factor H, FHL1, C4binding protein (C4BP) and also Plasminogen. The confocal image reveals that the bound proteins (shown in green) attach distant to the cell wall (shown in blue colour).

phages. CaCRASP2 bound to human CR3 receptor influences the host response for immune defense (Figure 2).

An additional, second immune evasion strategy, secretion of host complement degrading and inactivating proteases, was identified for *C. albicans*. In cooperation with Prof. B. Hube, Department Microbial Pathogenicity Mechanisms, we showed that *C. albicans* uses three secreted aspartic proteases (Saps) Sap1, Sap2 and Sap3 to degrade the human complement components C3b, C4b and C5 and to inhibit terminal complement complex (TCC) formation (Figure 3). This proteolytic activity is specific to the three Sap proteins. The triple knock out *C. albicans* strain Delta sap1-3 and also the non-pathogenic yeast *S. cerevisiae* lack such degrading activity.

Proteolytic degradation and inactivation of human complement components is a rather conserved mechanism and in collaboration with Prof. Axel Brakhage, Dept. Molecular and Applied Microbiology a very similar evasion strategy was shown for the human dermatophyte *A. benhamiae* and for *Aspergillus fumigatus*. The human dermatophyte *A. benhamiae* secretes proteases that degrade human complement components C3, C5, C6, and C7 and inhibit complement mediated hemolysis. For the human dermatophyte the timing of the two evasion strategies for controlling host complement attack was defined. The two mechanisms are relevant at different phases of infection. During the first and initial phase, i.e. immediately upon contact with human plasma and body fluids, the pathogen exploits the acquired human regulators and inhibitors for complement evasion (Figure 3). This immediate phase is relevant during the first seconds and minutes of infection and allows the pathogen to survive in the hostile environment. Then the second, the early phase is initiated. The pathogen secretes endogenous proteases that degrade host complement com-

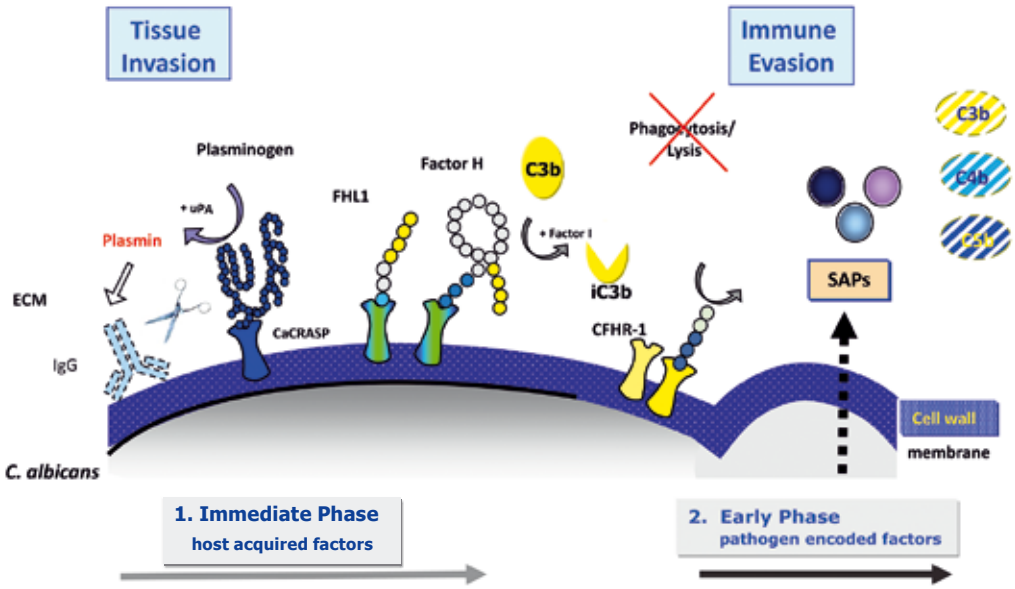
Figure 3

The human pathogenic yeast *Candida albicans* uses two separate mechanisms to inactivate human complement components.

The two mechanisms act at different phases of the infection. In the **immediate phase** of an infection (1, left panel) *Candida* acquires host regulators. These acquired host regulators inhibit complement and inactivate C3b. Bound plasminogen can be activated to the active protease plasmin that cleaves extracellular matrix components as well as immunoglobulins (IgG).

The second, **early phase** (2, right panel) is characterized by the secretion of aspartyl proteases (SAP). In particular SAP proteins (SAP1, SAP2 and SAP3) degrade the human complement proteins C3, C4 and C5 and block complement activation.

This type of combined immune evasion seems common to human pathogenic yeast and has also been detected for the dermatophyte *Arthroderma benhamiae* and *Aspergillus fumigatus*.



CRASPs - multipurpose tools of pathogen origin
SAPs - Secreted Aspartic Proteases

ponents and thus inactivate complement and inhibit formation of dangerous effector products (Figure 3).

Similarly to *Candida*, also the opportunistic human pathogenic fungus *Aspergillus fumigatus* secretes proteases that control complement activation and block release of damaging effector compounds. Culture supernatant derived from *A. fumigatus* efficiently cleaves the human complement components C3, C4, C5, C1q as well as immunoglobulin G. The *Aspergillus* encoded alkaline protease Alp1, which is secreted at high levels in the culture supernatant was identified as the central molecule responsible for this cleavage. The role of Alp1 was confirmed by generating an *alp1* deletion strain which exerts minimal complement degrading activity. Thus, *Aspergillus* Alp1 cleaves and degrades multiple host immune and complement effector components.

The human complement system and the newly generated activation compounds form a central and important immune barrier for pathogenic fungi, as well as for all infectious microbes and for microbial pathogens. Therefore any pathogen that aims to establish an infection and that aims to survive in an immunocompetent host has to cross and control this important immune barrier. The molecular identification of the multiple, timely separated evasion strategies highlights the complex interaction of pathogenic yeast and of the host immune response. The identification of microbial proteins that are central and relevant for infection and virulence provide interesting and promising candidates for immune intervention.

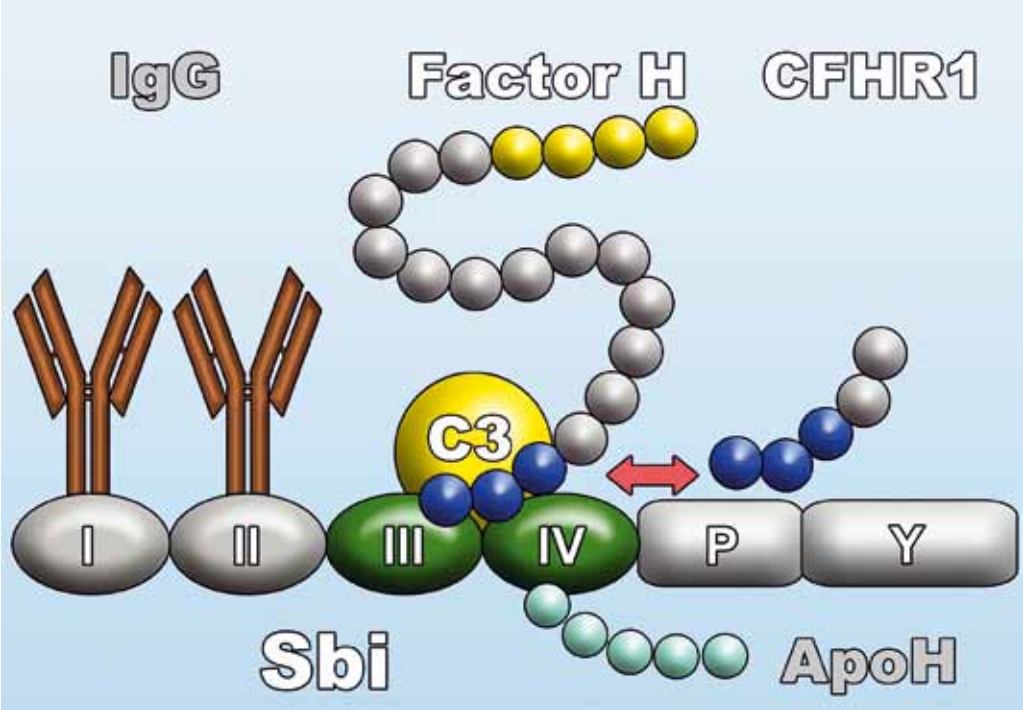


Figure 4
Sbi protein of the Gram positive bacterium *Streptococcus pyogenes* binds multiple host proteins.
Sbi (staphylococcal binder of immunoglobulin) binds multiple human soluble proteins such as IgG, ApoH and binds Factor H together with C3b to form a tripartite complex. Factor H is bound with the C-terminus together with C3b to Sbi domains III and IV.

2 Immune evasion of human pathogenic microbes

Group Leader: Peter F. Zipfel

Complement evasion is a common strategy used by multiple different pathogenic microbes including Gram positive bacteria, such as *Streptococcus pyogenes*, *S. pneumoniae and Staphylococcus aureus*, as well as Gram negative bacteria including *Borrelia burgdorferi*, related *Borrelia* species and *Pseudomonas aeruginosa*. The characterization of the various complement regulator binding proteins reveals patterns of similarity and identity, as well as diversity. The concept is emerging that individual CRAS proteins (complement regulator acquiring surface protein) that are derived from the various pathogenic bacteria and fungi bind several human plasma proteins and inhibitors including Factor H, FHL1, CFHR1, plasminogen, as well as extracellular matrix components such as fibronectin and fibrinogen at the same time.

The staphylococcal surface protein and secreted protein Sbi (staphylococcal binder of immunoglobulins) is a recently identified bacterial Factor H binding surface protein. Sbi also binds C3 and C3a, as well as immunoglobulins and the human plasma component beta2 glycoprotein (Figure 4). This bacterial surface protein binds Factor H together with C3 in a novel manner and forms a tripartite complex between Sbi, C3 and Factor H. Factor H together with C3b is bound to the staphylococcal protein via the C-terminal surface attachment region and is thus orientated in a way that leaves the N-terminal regulatory region exposed and accessible. This allows control and inhibition of complement activation and blockade of toxic complement effector functions at or close to the bacterial surface. Consequently, the pathogen can survive, evade and control host complement attack.

The emerging theme of redundancy shows that most pathogenic microbes, such as Gram negative, Gram positive bacteria, human pathogenic fungi, viruses as well as multicellular parasites express surface proteins that bind single human regulators. Thus a pathogenic microbe uses multiple Factor H-, as well as multiple plasminogen binding proteins. In addition individual microbial CRAS proteins are highly polymorphic which can be explained by antigenic variation.

CRAS proteins are identified in many pathogenic microbes and are likely present in all pathogens. Based on their binding characteristics for human complement inhibitors, CRAS proteins of such pathogenic microbes are divided into two groups. The first group, representing Type I proteins, bind Factor H and FHL1, but do not bind CFHR1. These proteins bind the two regulators via the major contact region, domain SCR 7. The second group, Type II proteins, bind Factor H and CFHR1, but do not bind FHL-1. Their major binding domain is contained in the C-terminal surface binding and attachment region of Factor H and CFHR1. Such unique binding characteristics are for example demonstrated for the type II *Borrelia* proteins CRASP3, CRASP4, and CRASP5. These three bacterial surface proteins bind the C3 convertase inhibitor Factor H and the terminal pathway inhibitor CFHR1 at the same time. Each of the three borrelial proteins has unique binding properties and in particular CRASP5 selects for CFHR1 and binds CFHR1 with higher intensity, as compared to Factor H. The effect of CRASP bound human regulators is at least two fold. The two human plasma proteins CFHR1 and Factor H bind simultaneously to the same site and compete for binding. Thus binding of CFHR1 is at the expense of Factor H mediated inhibitory activity on the C3 convertase level and for the gain of the CFHR1 mediated inhibitory effects on the C5 convertase and on the terminal pathway. This pattern of recruitment indicates that during bacterial infection also binding of the terminal pathway regulator CFHR1 is beneficial for the pathogen. Thus controlling and inhibiting the terminal phase of complement is relevant for infection.

3 Group ImmunoRegulation

Group Leader: Christine Skerka

The activated complement system exerts immune effector functions and modulates the intensity of the response in a self-controlling manner. An appropriate innate immune response is needed for recognition and removal of infectious agents and also for elimination of modified self cells.

Several complement regulatory proteins that are distributed in body fluids as well as anchored on self cell surfaces allow this type of self-control. The regulators control activation and progression of the cascade and ensure efficient inhibition of activated complement components on self tissues and further terminate complement activation. The importance of this regulation is reflected by the fact that several autoimmune diseases develop due to defective or absent complement regulators. Hemolytic Uremic Syndrome (HUS) and Membranoproliferative Glomerulonephritis (MPGN) are severe kidney diseases which are associated with impaired complement control. Similarly Age Related Macular Degeneration (AMD), a frequent disease in elderly people, is caused by defective complement regulation.

The aim of the research group ImmunoRegulation is to identify novel human complement regulators, to characterize their specific functions, and also to identify their role in autoimmune diseases, as well as their role upon infection.

The Factor H family includes the complement regulator Factor H and five additional Factor H related proteins (CFHR1 – CFHR5). All CFHR proteins are encoded by unique, separate genes, which are located in the *Factor H-CFHR* gene cluster, adjacent to the Factor H gene. The common feature of this family of secreted proteins is their exclusive composition of individual domains called short consensus repeats (SCR). The individual SCR domains of the various CFHR proteins show different degrees of sequence identity to each other and

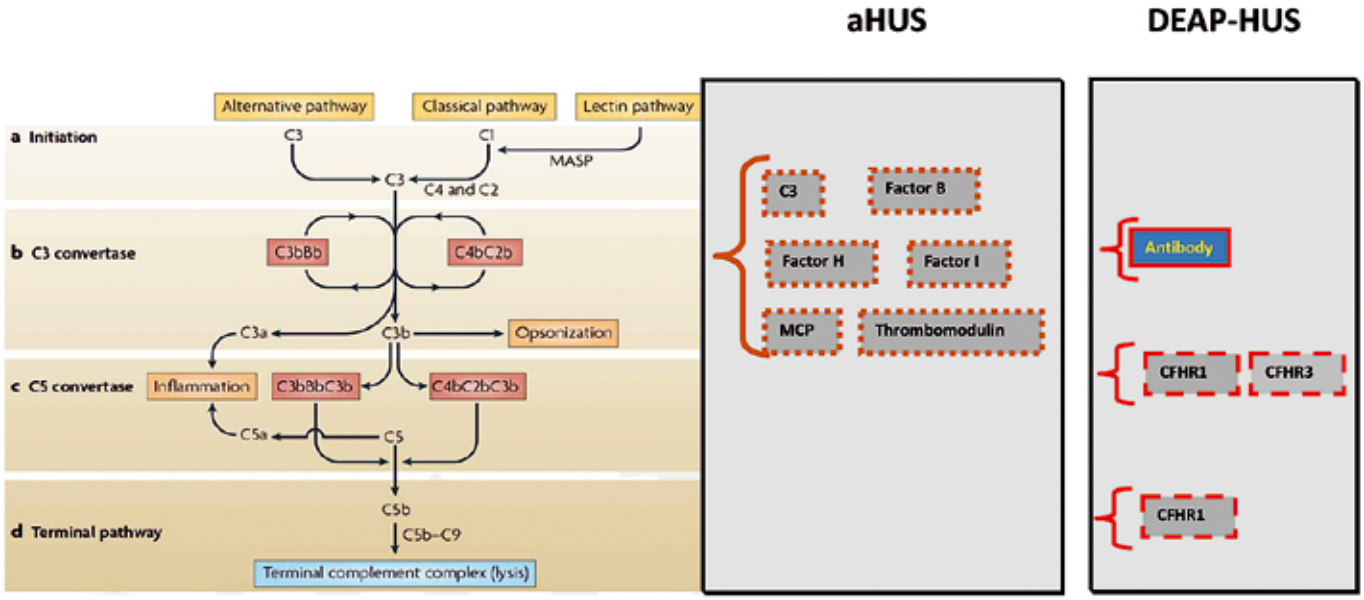


Figure 5
Gene mutations in different complement genes cause various subforms of Hemolytic Uremic Syndrome (HUS).
Gene mutations in several complement genes cause the renal disease Hemolytic Uremic Syndrome (HUS). The atypical form (aHUS) is associated with gene mutations that affect the regulators of the central complement convertase, such as Factor H, Factor I, MCP or thrombomodulin and also components of the convertase C3 and Factor B. The new form DEAP-HUS (deficient for CFHR plasma proteins and positive for autoantibodies to Factor H) is an autoimmune form where autoantibodies to Factor H are formed on the basis of a genetic deficiency. This autoimmune form is frequent in children and when properly diagnosed and treated has a rather good prognosis.

to Factor H, indicating complement regulatory functions. The function of each plasma protein in complement activation and regulation is currently poorly understood.

Interestingly a homozygous chromosomal deletion of the CFHR1 and CFHR3 genes, is a predisposing factor for the development of atypical HUS. In the Jena cohort of 147 HUS patients, 15 percent of the patients are homozygous deficient for CFHR1 and CFHR3, as compared to 2 % in healthy control cohort individuals. We have identified the chromosomal breakpoints in the genome of these patients and showed that a 84 kbp genomic fragment that is positioned downstream of the Factor H gene is deleted. About 75 % of patients with the homozygous deletion of CFHR1 and CFHR3 are also positive for autoantibodies to Factor H. This correlation shows that deficiency of CFHR1 and CFHR3 is a predisposing factor for the development of autoantibodies in HUS. The presence of the autoantibodies leads to the disease. Based on the common features of these patients this subgroup was termed DEAP-HUS (**de**ficient of CFHR proteins and **a**utoantibody **p**ositive, Figure 5). How exactly CFHR1 and CFHR3 deficiency causes a break of tolerance and enables autoantibody formation to Factor H is currently unclear and subject to further investigations.
As the chromosomal deletion results in a complete loss of CFHR1 and CFHR3 proteins as well as to generation of autoantibodies, our interest was to identify the function of CFHR1. CFHR1 is a novel human complement regulator, that inhibits the C5 convertase activity and that blocks the assembly of the terminal complement pathway (Figure 6). Thus the absence of CFHR1 in plasma due to deletion of the *CFHR1* gene results in a loss of complement regulatory activity (Figure 7).

These results have implications for diagnosis and therapy of DEAP-HUS patients, who need supplementation of the CFHR1 and CFHR3 pro-

Figure 6

Model of coordinated complement regulation by factor H and CFHR1 on the cell surface.

Factor H inhibits the C3 convertase C3bBb by decay activity as well as cofactor functions for proteolytic cleavage by the serine protease Factor I. Factor H binds to C3b and to self cell surfaces. CFHR1 acts downstream of factor H and blocks the C5 convertase C3bBbC3b by inhibition of C5 activation to C5b and C5a, as well as deposition of C5b6(7) to the membrane. CFHR1 binds to C3b and self cell surfaces and competes with factor H for binding sites.

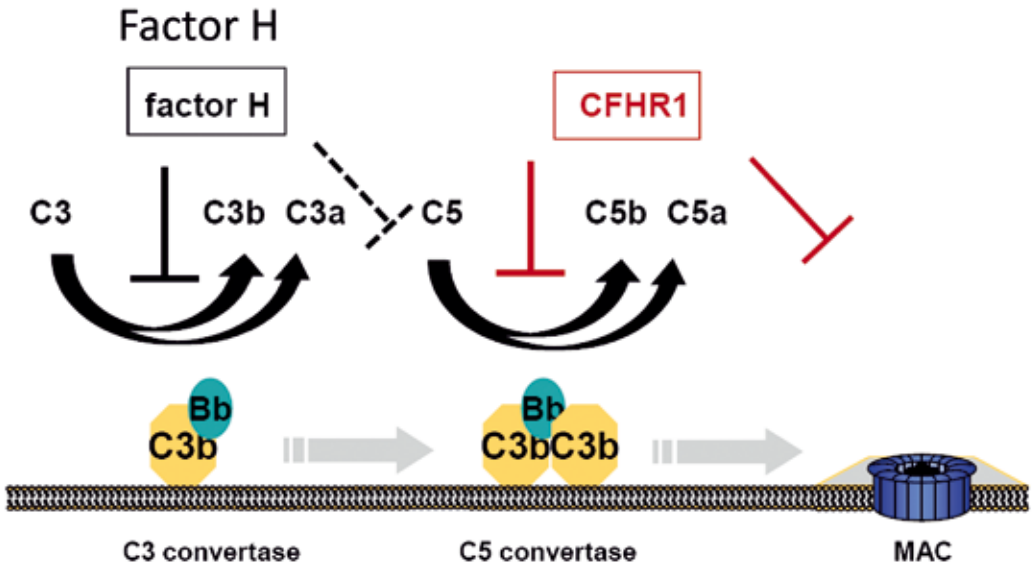
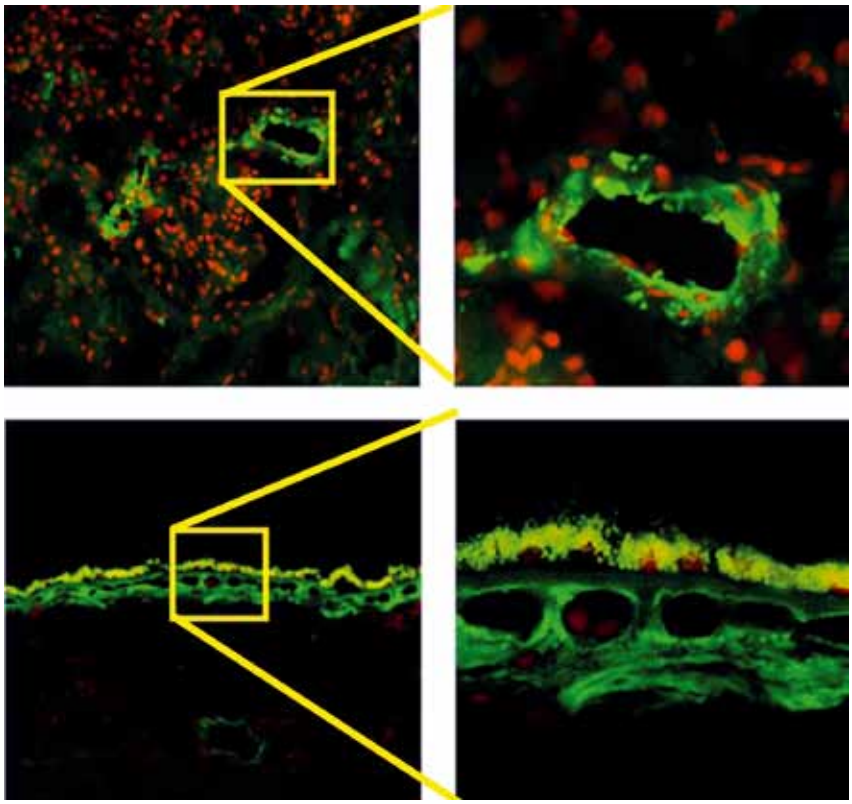


Figure 7

Expression of CFHR1 in renal and retinal tissue.

Immunofluorescence staining of CFHR1 (green fluorescence) in renal and retinal human tissue. CFHR1 is detected at the lining of renal (upper panels) and occular (lower panels) blood vessels including large arteries, afferent and efferent arterioles associated with glomeruli (upper panel), or the choriocapillaries (lower panel) as well as Bruch's membrane (nuclear counterstain: propidium iodide; original magnification ×100). Thus CFHR1 is present at the surface of endothelial cells and at the Bruch's membrane. Autofluorescence of lipofuscin containing cells appears yellow.



teins in combination with immune suppressive therapy to reduce autoantibody titers. The results were directly translated into therapy and several patients were treated in collaboration with docent Dr. Müller from the Department of Pediatric Nephrology, Charité in Berlin.

Also the gene coding for CFHR2, an additional member of the Factor H family is associated with a renal disease with Membranoproliferative Glomerulonephritis type II. An MPGN patient was identified who showed a chromosomal rearrangement in the Factor H gene locus, that resulted into a duplication of the CFHR2 gene. In order to determine the physiological relevance of this duplication the CFHR2 protein was recombinantly expressed and used for detailed functional characterization. Recombinant CFHR2 binds to the central complement component C3b and to heparin. This binding preference indicates a role of CFHR2 as human complement regulator, that likely also differentiates between self and non self surfaces. Further characterization of CFHR2 will define the specific role of this protein.

4 Complement dysfunction in human diseases

Group Leaders: Christine Skerka, Peter F. Zipfel

Complement has a major and central role in maintaining tissue and cell integrity and homeostasis. Consequently genetic modifications of central complement inhibitors and components affect this delicate balance and lead to disease.

Several genetic scenarios, including gene mutations, gene deletion and copy number variations of complement genes result in human diseases. Gene mutations in the Factor H gene develop into multiple disorders manifesting in the kidney such as Hemolytic Uremic Syndrome (HUS) and Membranoproliferative Glomerulonephritis type II (MPGN II) or in the retina of the eye causing Age Related Macular Degeneration (AMD).

Factor H gene mutations are frequently observed in the rare disorder **Atypical Hemolytic Uremic Syndrome** (aHUS). For this disease, the identified mutations cluster in the C-terminal surface recognition and attachment region of the Factor H protein and the majority of mutations occur in heterozygous setting. This genetic predisposition shows for the majority of patients one intact and one defective allele resulting in reduced Factor H plasma and functional levels. Apparently these conditions result in almost normal fluid phase complement control, but in situations of immune stress and local cell damage when soluble regulators are required at the cell surface, the defective function or reduced protective level affects tissue and cell homeostasis (Figure 8). We have established a large registry for atypical HUS patients and a European based registry for the autoimmune form DEAP-HUS.

Factor H gene mutations in the severe renal disease **Membranoproliferative Gomerulonephritis type II** (MPGN II) affect both alleles and most frequently cause a block of protein secretion. Defective Factor H secretion is frequently reported for mutations which affect central, architectural relevant Cys residues. Exchange of these conserved residues causes defective protein folding and intracellular processing and leads to block of protein secretion. Most of the patients lack Factor H in plasma and this results in uncontrolled complement activation in the fluid phase, consumption of C3 and low levels of active C3 molecules in the circulation. Patients develop kidney damage due to thickening of the glomerular basement with deposits within the lamina densa of the glomerular basement membrane. In addition to several human cases this type of mutation has been confirmed in animal models, such as Factor H deficient pigs, which represent natural mutants and the first animal model for MPGN, and in Factor H knock out mice, which were genetically designed.

A European MPGN registry was established, which includes more than 50 MPGN patients from different European countries. About 65 % of the individuals show defective complement activation or control and genetic

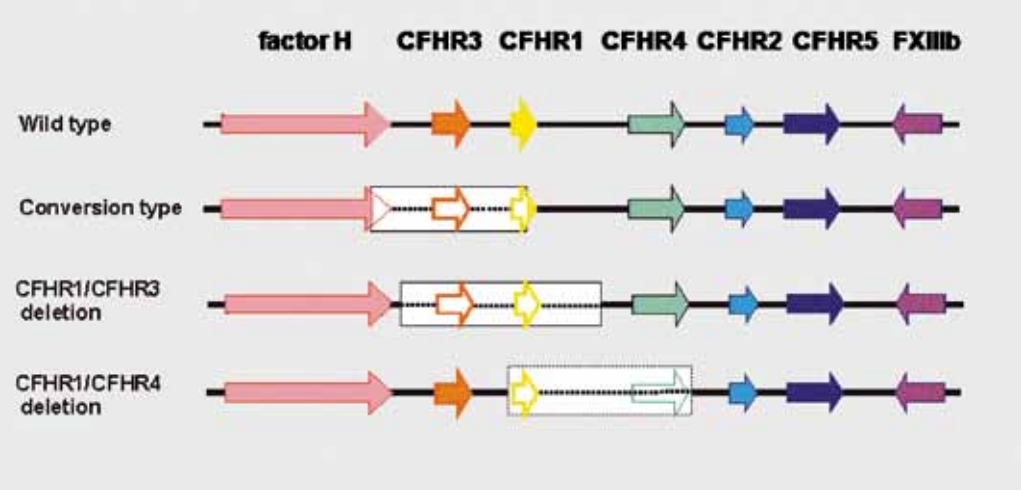


Figure 8
Chromosomal deletion of a 84 kbp fragment in the Factor H gene.
Genetic deletion of the two genes in the Factor H gene cluster *CFHR1* and *CFHR3* are associated with the kidney disease HUS, but at the same time show a protective effect for AMD. Different genetic variants were identified which show that non homologues recombination causes deletion in the Factor H gene cluster on human chromosome 1.

analysis of the Factor H gene revealed one mutation in two sisters from a consanguineous family who lack one single amino acid in SCR4 of Factor H (ΔLys224) which is contained in the complement regulatory region of the protein and which affects the complement inhibitory functions. In addition two sequence variations were identified in the Factor B gene which are more frequent in the MPGN cohort as compared to the control group. Similarly a novel allele was identified in the gene coding for CFHR1. This particular SNP occurs at low levels and is detected with similar frequencies in the MPGN cohort and in the control group (Figure 6). In summary complement genes are associated with severe renal diseases and apparently the fine tuned regulation of complement in renal tissue is important for tissue homeostasis. Defective regulation at levels of the C3 and C5 convertase apparently affects homeostasis and develops into pathology.

For the retinal disease **Age Related Macular Degeneration** (AMD), which is a common form of blindness in the elderly population of the Western World, a common polymorphism, resulting in the exchange of a Tyrosine to Histidine residue at position 402 of Factor H is associated with the disease.

The various Factor H mutations and amino acid exchanges within this multifunctional protein cause defects that manifest in different organs, like the kidney and the retina. Thus complement Factor H plays a central role for organ and cell homeostasis and different regions of this multifunctional protein are required for local activity in different organs. In summary mutations or chromosomal deletions within the Factor H-CFHR gene cluster affect local control of complement at surfaces and result in autoimmune diseases. Understanding the molecular processes in complement regulation helps to define the underlying pathomechanisms of diverse diseases

including HUS, MPGN and AMD. This allows to establish novel diagnostic approaches and new concepts for therapy.

5 Role of complement and of Factor H in apoptosis

Group Leader: Peter F. Zipfel

Every day billions of cells are generated in any vertebrate as part of development, tissue remodeling, cellular senescence or as non productive or selected immune cells that need to be eliminated. Efficient clearance and rapid elimination of such unwanted cells, cellular debris and particles is central for homeostasis. Complement has a major role in the recognition and removal of modified self cells, including necrotic cells, as well as apoptotic cells and apoptotic particles (Figure 1). Apoptotic cells, apoptotic particles, as well as necrotic debris and also cytoplasmic components released during the process of necrosis, need to be eliminated in a fast, safe and non inflammatory manner. Rapid and efficiently removal of such material occurs by phagocytosis. Complement is central in these steps as complement tags modified self cells and material to enhance phagocytosis in an immunologically silent, efficient and non inflammatory manner.

Recent evidence shows that the complement regulator Factor H is actively involved in the removal of cellular debris and modified self cells. Factor H binds specifically to the surface of apoptotic particles or necrotic cells and assists in the efficient removal of such particles. This process apparently requires a fine tuned and regulated activation of the complement cascade to the level of C3 convertase. This activation leads to controlled and moderate activity of C3 convertase to allow opsonization of altered self surfaces and particles with C3b and C3d. Surface bound C3d enhances opsonization and uptake by phagocytic cells. For these regulated events Factor H inhibits downstream steps and to ensure that further activation and progression of the C5 convertase and of the terminal pathway is restricted. Control at this level ensures efficient opsonization and blocks generation of inflammatory mediators, such as anaphylactic peptide C5a.

Factor H mediated clearance and removal of modified and altered self material is further affected and controlled by the acute phase reactant C-reactive protein (CRP). Factor H binds in a calcium independent manner to monomeric CRP (mCRP), but not to pentameric CRP (pCRP). Factor H has three major binding sites for mCRP. We recently identified an additional, novel binding site. This third binding site is located within the C-terminal SCRs 18-20, that forms the central recognition and cell attachment site of Factor H. On the surface of altered self cells, phosphorylcholine represents a major ligand for mCRP and mCRP directs Factor H to the surface of damaged and modified human cells, like apoptotic endothelial cells. Factor H-mCRP complexes enhance C3b inactivation both in fluid phase and in particular on the surface of damaged cells and inhibit production of pro-inflammatory cytokines like TNF-α and IL-8. By recruiting the soluble complement inhibitor Factor H to the surface of damaged cells, mCRP inhibits progression of the complement cascade beyond the C3 convertase level and prevents formation of inflammatory activation products. Thus mCRP-Factor H complexes contribute to the safe removal of opsonized damaged cells and particles.

6 Technical Support Unit

Group Leader: Susanne Lorenz

Technical Support Unit for Media Production and Waste Management

The Technical Support Unit provides media and basic research material for all departments and research groups of the institute. In addition, waste management for inactivation of hazardous biological compounds is a major area of responsibility of this unit.

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Deutsche Forschungsgemeinschaft
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Deutsche Forschungsgemeinschaft
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Christine Skerka

National Institutes of Health
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Targeted Complement Inhibition as a Therapeutic Approach for Dense Deposit Disease
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Pro Retina Stiftung
Die Rolle des Komplementsystems, insbesondere der beiden Komplementregulatoren Faktor H und FHL-1, bei der AMD des Auges
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Selected publications
(HKI authors in bold)

Haupt K, **Reuter M**, van den Elsen J, Burman J, **Hälbich S**, **Richter H**, **Skerka C**, **Zipfel PF** (2008) The *Staphylococcus aureus* protein Sbi acts as a complement inhibitor and forms a tripartite complex with host complement Factor H and C3b. *PLoS Pathogens* 4, e1000250.

Józsi M, **Zipfel PF** (2008) Factor H family proteins and human diseases. *Trends in Immunology* 29, 380-387.

Ståhl AI, Vaziri-Sani F, **Heinen S**, Kristoffersson AC, Gydell KH, Raafat R, Gutierrez A, Beringer O, **Zipfel PF**, Karpman D (2008) Factor H dysfunction in patients with atypical hemolytic uremic syndrome contributes to complement deposition on platelets and their activation. *Blood* 111, 5307-5315.

Castelletti F, Donadelli R, Banterla F, Hildebrandt F, **Zipfel PF**, Bresin E, Otto E, **Skerka C**, Renieri A, Todeschini M, Caprioli J, Caruso MR, Artuso R, Remuzzi G, Noris M (2008) Mutations in FN1 cause glomerulopathy with fibronectin deposits. *Proc Natl Acad Sci USA* 105, 2538-2543.

Józsi M, Licht C, **Strobel S**, **Zipfel SLH**, **Richter H**, **Heinen S**, **Zipfel PF**, **Skerka C** (2008) Factor H autoantibodies in atypical hemolytic uremic syndrome correlate with CFHR1/CFHR3 deficiency. *Blood* 111, 1512-1514.

Gropp K, **Schild L**, **Hube B**, **Zipfel PF**, **Skerka C** (2009) The yeast *Candida albicans* evades the human complement system by secretion of aspartic proteinases. *Mol Immunol* 47, 465-475.

Heinen S, **Hartmann A**, **Lauer N**, **Wiehl U**, **Dahse HM**, Schirmer S, **Gropp K**, **Enghardt T**, Wallich R, **Hälbich S**, **Mihlan M**, Schlötzer-Schrehardt U, **Zipfel PF**, **Skerka C** (2009) Factor H related protein 1 (CFHR-1) inhibits complement C5 convertase activity and terminal complex formation. *Blood* 114, 2439-2447.

Licht C, Pluthero FG., Li L, Christensen H, Habbig S, Hoppe B, Geary DF, **Zipfel PF**, Kahr WHA (2009) Platelet-associated complement Factor H in normal individuals and patients with atypical HUS. *Blood* 114, 4538-4545.

Mihlan M, **Stippa S**, **Józsi M**, **Zipfel PF** (2009) Monomeric CRP contributes to complement control in fluid phase and on cellular surfaces and increases phagocytosis by recruiting Factor H. *Cell Death and Diff* 16, 1630-1640.

Zipfel PF, **Skerka C** (2009) Complement regulators and inhibitory proteins. *Nature Reviews Immunology* 10, 729-740.

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Department of Microbial Pathogenicity Mechanisms

Department of Microbial Pathogenicity Mechanisms

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

The scientific work of MPM includes several projects based on close collaborations with other departments of the Hans Knöll Institute and a number of established national and international research groups.

Members and projects of MPM are supported by several national and international programmes and research funding organisations such as: Netzwerk Grundlagenforschung (of the HKI), Jena School for Microbial Communication (JSMC), International Leibniz Research School (ILRS), Deutsche Forschungsgemeinschaft (DFG), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES, Brasil), Studienstiftung des Deutschen Volkes, Federal Ministry of Education and Research (BMBF), and the Framework 7 programme of the European Union. Projects are integrated into networks such as the FunPath consortium, the FINSysB network, or the DFG Priority Programme SPP1160 "Colonisation and Infection by Human Pathogenic Fungi". Finally, research is supported in parts by companies such as Bayer Vital GmbH or Biolitec AG.

Candida infections

In contrast to most pathogenic fungi of humans, such as *Aspergillus fumigatus*, *Cryptococ-*

INTRODUCTION | EINLEITUNG

Head:
Prof. Dr. Bernhard Hube

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

Die wissenschaftliche Arbeit der Abteilung MPM beinhaltet verschiedene Projekte und basiert auf engen Kooperationen mit anderen Abteilungen des Hans-Knöll-Instituts und etablierten nationalen und zahlreichen internationalen Forschungsgruppen.

Die Mitglieder und deren Projekte werden von verschiedenen nationalen und internationalen Programmen und Forschungsförderungsinstitutionen unterstützt, wie beispielsweise durch das Netzwerk Grundlagenforschung (HKI), die Jena School for Microbial Communication (JSMC), International Leibniz Research School (ILRS), die Deutsche Forschungsgemeinschaft (DFG), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES, Brasilien), die Studienstiftung des Deutschen Volkes, das Bundesministerium für Bildung und Forschung (BMBF) und Framework 7, ein Programm der Europäischen Union. Die Projekte sind integriert in Netzwerke wie die FunPath-Initiative, FinSysB oder das DFG-Schwerpunktprogramm SPP1160 "Colonisation and Infection by Human Pathogenic Fungi". Teilweise werden die Projekte auch durch Firmen wie Bayer Vital GmbH und Biolitec AG unterstützt.

Candida-Infektionen

Im Gegensatz zu vielen anderen krankheitserregenden Pilzen des Menschen, wie zum Beispiel *Aspergillus fumigatus*, *Cryptococcus neoformans* und *Histoplasma capsulatum*,

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

An intact immune system and a balanced microbial flora are normally sufficient to protect an individual from *Candida* infections. However, certain critical events such as extensive antibacterial treatment or immune system dysfunction may enable these fungi to overgrow the microbial flora on mucosal surfaces (superficial candidosis). In hospital settings, *Candida* species may cause life-threatening systemic and invasive infections in a growing population of vulnerable patients. In fact, bloodstream infections due to *Candida* (candidaemia) are associated with the highest crude mortality rate of all bloodstream infections, even higher than those caused by bacteria (Perlroth *et al.*, 2007). *Candida* cells may enter the bloodstream by direct penetration from epithelial tissues, due to damage of barriers in the body caused by surgery, polytrauma or drug treatment, or may spread from biofilms produced on medical devices (e. g. catheter). From the bloodstream, cells can infect every organ of the body (invasive candidosis), but appear to prefer certain organs depending upon

welche normalerweise nur in der Umwelt zu finden sind, zählen *Candida albicans* und *Candida glabrata* zur normalen mikrobiellen Flora der Haut, des Verdauungstraktes oder anderer Oberflächen mit Schleimhäuten, und gelten in der Regel als harmlose Kommensale. Tatsächlich können diese kommensalen Hefen bei bis zu 71 % der gesunden Bevölkerung gefunden werden, abhängig von den Untersuchungsmethoden und der Körperregion (Ruhnke, 2001).

Ein intaktes Immunsystem und eine gesunde mikrobielle Flora sind normalerweise ausreichend, um einen Menschen vor einer *Candida*-Infektion zu schützen. Unter bestimmten Umständen, zum Beispiel nach antibakteriellen Behandlungen oder bei Fehlfunktionen des Immunsystems, kann der Pilz die normale mikrobielle Flora auf Schleimhautoberflächen überwuchern (oberflächliche Candidosen). In einer stetig anwachsenden Anzahl von gefährdeten Patienten, die in Krankenhäusern behandelt werden, können *Candida*-Arten sogar lebensbedrohliche systemische und invasive Infektionen verursachen. Tatsächlich ist die Letalitätsrate bei Blutinfektionen durch *Candida* (Candidämie) höher als bei bakteriellen Infektionen (Perlroth *et al.*, 2007). *Candida*-Zellen können das Blutgefäß-System durch direkte Penetration von den Epithelschichten, nach Zerstörung physikalischer Barrieren durch chirurgische Eingriffe, Polytrauma oder medikamentöse Behandlungen, oder durch Verbreitung ausgehend von Biofilmen auf implantierten medizinischen Hilfsmitteln (z. B. Katheter), erreichen. Von den Blutgefäßen aus können die Pilzzellen praktisch jedes Organ des Menschen infizieren (invasive Candidosen), sie scheinen jedoch, je nach Infektionsroute, bestimmte Organe zu bevorzugen (Kullberg *et al.*, 2002).

the route of infection (Kullberg *et al.*, 2002). Only relatively minor immunosuppression is required to predispose to invasive *Candida* infections, and *Candida* is by far the most common cause of nosocomial (hospital acquired) fungal infections (Perlroth *et al.*, 2007).

Candida albicans

The yeast *Candida albicans* is regarded as the most important of all medically relevant fungi and is an extremely successful pathogen of human beings. *C. albicans* is a polymorphic yeast which belongs to the normal microbial flora of human beings. Oral and vaginal infections with *C. albicans* are extremely common in even mildly immunocompromised individuals. Furthermore, in severe cases, *C. albicans* penetrates into deeper tissue and may enter the bloodstream. From the bloodstream, the fungus has the potential to invade almost all body sites and organs, causing life-threatening systemic infections that require the adaptation to a variety of different environmental stresses. Therefore, *C. albicans* is able to survive and proliferate in radically changing environments with drastic changes in oxygen and carbon dioxide, pH, osmolarity, availability of nutrients and temperature. In addition it has to resist the constant surveillance of the immune system. Few pathogenic microorganisms have the potential to cause infections at such a broad range of body sites.

The availability of the *C. albicans* genome sequence and whole-genome microarrays,

Bereits relativ geringe Immunosuppressionen reichen aus, um einen Menschen für eine invasive Candidose empfänglich zu machen und *Candida* gilt als der bei weitem häufigste Erreger von nosokomialen (im Krankenhaus erworbenen) Pilzinfektionen (Perlroth *et al.*, 2007).

Candida albicans

Candida albicans gilt als der medizinisch wichtigste Pilz und ist ein extrem erfolgreicher Pathogen des Menschen. *C. albicans* ist eine polymorphe Hefe, die zur normalen mikrobiellen Flora des Menschen gehört. Selbst bei Menschen mit einem nur gering geschwächten Immunsystem kommen häufig orale und vaginale *C. albicans*-Infektionen vor. Bei stärkeren Störungen kann es dazu kommen, dass *C. albicans* in tiefere Gewebeschichten vordringt und von dort sogar das Blutsystem erreicht. Aus dem Blut kann *C. albicans* in die meisten Organe eindringen und so lebensbedrohliche systemische Infektionen hervorrufen. Während dieses Prozesses muss sich *C. albicans* an unterschiedlichste Umweltbedingungen anpassen – dazu gehören Schwankungen im Sauerstoff- und Kohlendioxidgehalt, dem pH-Wert, der Osmolarität, den verfügbaren Nährstoffen und der Temperatur. Außerdem muss der Pilz den Angriffen des Immunsystems widerstehen. Nur wenige Mikroorganismen sind wie *C. albicans* in der Lage, an so vielen verschiedenen Stellen des Körpers Infektionen hervorzurufen.

Durch die Verfügbarkeit der Sequenz des *C. albicans*-Genoms und von genomweiten Mikroarray-Chips, sowie der Entwicklung von Techniken, die relativ einfach schnelle molekularbiologische Manipulationen des *C. albicans*-Genoms ermöglichen, wurden in den

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

Candida glabrata

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

Three attributes make *C. glabrata* an attractive model organism for fungal infections: *C. glabrata* is non-filamentous, has a haploid genome and is very closely related to the non-pathogenic baker's yeast *Saccharomyces* cerevisiae. In fact, the sequencing of the *C. glabrata* genome uncovered many striking similarities to *S. cerevisiae* (Dujon *et al.*, 2004). Many genomic and molecular biology tools such as promoters, markers, reporters and even plasmids are interchangeable between the two yeasts.

letzten Jahren eine Fülle von Informationen über die faszinierende Biologie und die Virulenzmechanismen dieses Pathogens generiert (Berman *et al.*, 2002). Darüber hinaus wurde eine Reihe von Infektionsmodellen etabliert, um genomweite Genexpressionen oder die Beteiligung von ausgesuchten Genen bei Infektionen untersuchen zu können (Zakikhany *et al.*, 2007; Schaller *et al.*, 2006; Thewes *et al.*, 2007).

Candida glabrata

C. glabrata gilt als "emerging pathogen", der immer häufiger in Kliniken isoliert wird. In vielen Fällen ist *C. glabrata* nach *C. albicans* mittlerweile die zweithäufigste pathogene Hefe, die beim Menschen gefunden wird. Ein wichtiger Faktor dabei dürfte die natürliche hohe Resistenz gegen die meisten gebräuchlichen Antimykotika sein. Trotzdem gibt es relativ wenig Studien, die sich mit den Pathogenitäts-Mechanismen von *C. glabrata* befassen.

Drei Eigenschaften machen *C. glabrata* zu einem attraktiven Modellorganismus für Pilzinfektionen: *C. glabrata* ist ein nicht-filamentöser Pilz, hat ein haploides Genom und ist ein naher Verwandter der klassischen Bäckerhefe *Saccharomyces cerevisiae*. Die Sequenzierung des *C. glabrata*-Genoms brachte verblüffende Ähnlichkeiten mit *S. cerevisiae* zutage (Dujon *et al.*, 2004). Viele genomische und molekularbiologische Werkzeuge, wie z. B. Promotoren, Markergene, Reportergene und selbst Plasmide können in beiden Hefen verwendet werden. Die bekannte Genomsequenz von *C. glabrata*, eine Transposon-Insertions-Bibliothek und Mikroarray-Chip-Techniken, bilden eine solide Basis für die molekulare Analyse der Pathogenitätsmechaismen.

The known genome sequence of *C. glabrata*, a transposon insertion library and microarray techniques provide a solid basis for advanced molecular analysis of pathogenicity traits and mechanisms.

Analysis of the pathogenicity mechanisms of *Candida*

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

Infection models for fungal infections

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

Infection models in mice are routinely used to study virulence of *Aspergillus fumigatus* and *Candida albicans* mutant strains. Murine models for *A. terreus* and *C. glabrata*, two additional fungal pathogens studied by groups of the HKI, are further developed. Survival is the traditional readout parameter for most *in vivo* models; however, in depth post mortem analysis is necessary to dissect the underlying mechanisms leading to attenuation of defined mutants. Histopathology is a useful way to gain information on the cellular pro-

Untersuchungen zu Pathogenitätsmechanismen von *Candida*

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

Infektionsmodelle zur Untersuchung pathogener Pilze

Eingebettet in die Abteilung MPM ist die Forschungsgruppe Infektionsmodelle und Wirkstoffprüfung. Forschungsschwerpunkte dieser Gruppe liegen auf der experimentellen Infektionsbiologie.

Zur Untersuchung der Virulenz von *Aspergillus fumigatus* und *Candida albicans* setzt die Gruppe etablierte Mausmodelle ein. Murine Infektionsmodelle für *A. terreus* und *C. glabrata*, zwei weitere humanpathogene Pilze, an denen am HKI geforscht wird, werden zur Zeit weiterentwickelt. Traditionell wird die Virulenz von Pilzmutanten anhand der Mortalität infizierter Versuchstiere beurteilt. Um jedoch zu verstehen, warum bestimmte Mutationen die Virulenz vermindern, sind tiefergehende Untersuchungen notwendig. Mittels Histopathologie können auf zellulärer Ebene Einblicke in den Infektionsprozess gewonnen werden. In Kooperation mit dem am Beutenberg-Campus ansässigen Fritz-Lipmann-Institut werden aus Gewebeproben histologische Präparate angefertigt, die dann von der Gruppe ausgewertet

cesses during infection. In collaboration with the Fritz-Lipmann-Institute (FLI) at the Beutenberg Campus we process tissue samples for histopathology. Additionally, organ damage is monitored by measuring blood levels of marker enzymes.

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

A tool to reduce and refine animal models is *in vivo* imaging. It can be used to directly monitor the progression of infections and inflammation in animals over time. We are currently establishing positron emission tomography/computer tomography (PET/CT) to study the progression and magnitude of inflammation and fungal burden during invasive aspergillosis and disseminated candidiasis in mice.

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

References

Berman J et al. (2002) *Nat Rev Genet* 3, 918–930.

Dujon B et al. (2004) *Nature* 430, 35–44.

Kullberg BJ and Oude Lashof AML (2002) *Eur J Med Res* 7, 183-191.

Perlroth J et al. (2007) *Med Mycol* 45, 321–346.

Ruhnke M (2001) Ed. *ASM Press*, 307–325.

Schaller M et al. (2006) *Nat Protoc* 1, 2767–2773.

Thewes S et al. (2007) *Mol Microbiol* 63, 1606–1628.

Zakikhany K et al. (2007) *Cell Microbiol* 9, 2938–2954.

werden. Als zusätzliche Informationsquelle über die Prozesse *in vivo* werden Parameter der klinischen Chemie und Blutenzymwerte herangezogen.

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

Eine Möglichkeit, um Tierversuche zu verringen und zu verbessern, ist die bildliche *in vivo*-Darstellung. Dadurch kann der Infek-tionsverlauf direkt überwacht werden. Zur Zeit etablieren wir eine Methode der Positronen-Emissions-Tomograpie/Computer-Tomography (PET/CT), um Verlauf und Ausmaß der Entzündung sowie den Pilzbefall während invasiver Aspergillose und Candidiasis zu untersuchen.

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

1 *Candida albicans* interactions with human epithelial cells

Group Leaders: Betty Wächtler, Bernhard Hube

The yeast *Candida albicans* is both, a harmless commensal and an aggressive pathogen. The fungus frequently causes superficial infections by invading and damaging epithelial cells, but may also cause systemic infections by penetrating through epithelial barriers. During the most common type of oral infections – oropharyngeal candidosis, the fungus invades the oral mucosa and persists within the epithelium causing superfical lesions. However, the main reservoir of *C. albicans* is the gastro-intestinal tract, and systemic infections predominantly originate from this niche. Within healthy individuals, fungal proliferation, colonization and invasion are hindered by dense epithelial layers with high turnover rates and innate defence mechanisms such as secretion of antimicrobial compounds. However, *C. albicans* has developed strategies to overcome these defence mechanisms for both commensal growth and infection. One of the strategies includes the morphological switch from yeast cells to filamentous hyphae and the virulence attributes associated with the transition.

In this project we study the interactions between *C. albicans* and oral epithelial cells as well as enterocytes during early infections to identify mechanisms, by which *C. albicans* enters these cells. One already described process during interaction with epithelial cells is the induction of its own endocytosis. In this process, the hyphal associated GPI-protein Als3 plays a key role in the up-take of the fungus by epithelial or endothelial cells via the host receptors E- and N-cadherin (Phan *et al.*, 2007). A second mechanism is an active physical penetration, supported by extracellular hydrolytic activity of the fungus. *In vitro* it is possible to clearly differentiate between these two processes and in our studies we have begun to search for fungal factors which are involved in one or both routes of invasion. In collaboration with Frederic Dalle (University of Dijon) we examined the interactions between *C. albicans* and epithelial cells (cell line TR-146) and enterocytes (cell line Caco-2). The fungus is able to adhere to, invade into and damage these cells, however, to a different extent. This indicates that adhesion, invasion and damage by *C. albicans* depends on the host cell type and the differentiation stage of epithelial cells, demonstrating that host cells differ in their susceptibility to the fungus (Dalle *et al.*, 2009). Furthermore, invasion into oral epithelial cells can occur via both distinct invasion routes: Induced endocytosis significantly contributes to early stages of invasion, while the major route at later stages is active penetration. In contrast, invasion into enterocytes occurs via active penetration only (Dalle *et al.*, 2009). Surprisingly, secreted aspartic proteases (Saps) of *C. albicans* seem to contribute to both induced endocytosis and active penetration.

Furthermore, we investigated the molecular basis and further dissected the stages of *C. albicans* interactions with epithelial cells by using a series of selected fungal mutants. Analysis of mutants defective in certain factors and/or activities showed that adhesion relies on regulators of hyphal formation, hyphal orientation/thigmotropism and cell wall integrity, a vacuolar sorting protein, and cell surface adhesins. Overall adhesion was found to be an important activity for subsequent invasion, as most mutants with reduced adhesion properties also showed reduced invasion and damage of epithelial cells. All steps of interaction required regulators of hyphal development, whereas hyphal orientation/thigmotropism, glycerol content, glycosylation, cell wall integrity and hyphal extension were specifically required

References

Dalle F et al. (2009) *Cell Microbiol* 12, 248–271.

Phan QT et al. (2007) *PLoS Biol* 5, e64.

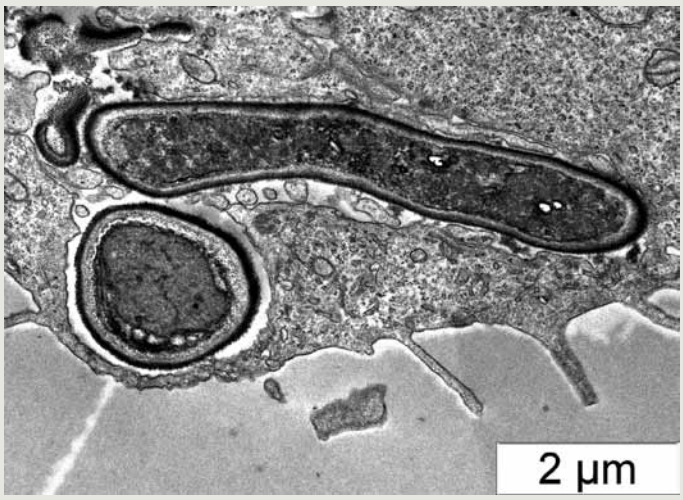

Figure 1
Transmission electron microscopic picture (TEM) of epithelial cells invaded by viable *C. albicans* hyphae. The fungal cells are tightly surrounded by the epithelial membrane and the epithelial cells show typical membrane ruffles.

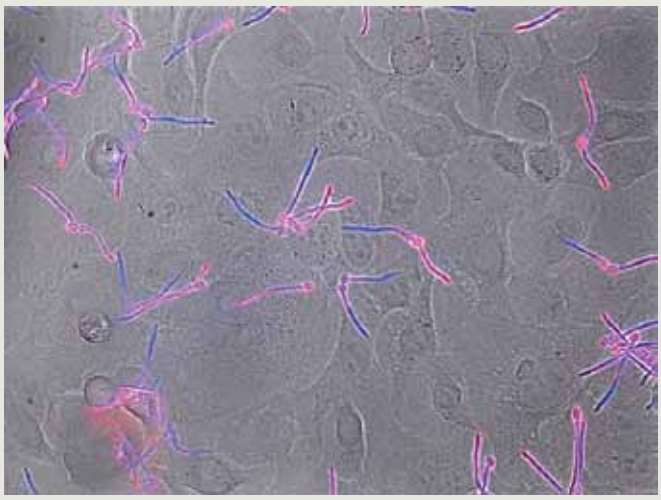

Figure 2
Overlay picture of the interaction of *C. albicans* with oral epithelial cells. Invasion of *C. albicans* hyphae is clearly visible after differential staining. Bright field shows the epithelial and *C. albicans* cells. Fluorescence staining in red (ConA) after immunodetection of *C. albicans* before permeabilisation and in blue (CW) after permeabilisation for all *C. albicans* cells (extracellular and internalised).

for invasion and damage. Moreover, host serum factors influence adhesion, invasion and damage, whereas several tested extracellular matrix proteins have only a minor effect on these processes. Finally, invasion was not restricted to the initial invaded host cell and hyphae frequently penetrated adjacent cells, which can be considered as an additional stage of invasion. Therefore, distinct sets of fungal genes, factors and activities are necessary for the individual stages of *C. albicans* interacting with epithelial cells from attachment to invasion and to damage.

2 Functional analysis of genes associated with oral candidosis

Group Leaders: Ronny Martin, Bernhard Hube

In this DFG-funded project (Hu528/10 "Identification and characterisation of virulence associated genes during oral infections with *Candida albicans*" within the priority progamme SPP 1160 "Colonisation and infection with human pathogenic fungi") we have analysed the expression of *C. albicans* genes during experimental oral infection. To this end, we have used a model based on reconstituted human oral epithelium (RHE), a multilayer of the cell line TR146, which mimics epithelial tissue of the oral cavity (for details see Schaller *et al.*, 2006). During infections of RHE with *C. albicans* we have identified three distinct stages: (i) attachment of fungal cells to the surface of the host tissue, (ii) invasion of *C. albicans* germ tubes into the superfical cell layer and (iii) dissemination of hyphae into the deeper parts of the tissue which correlated with strong increasement of cell damage and ended in tissue destruction (Zakikhany *et al.*, 2007). Transciptional profiling of *C. albicans* during RHE infection identified stage-specific gene expression. Attachment was characterized by expression of genes involved

in the morphological change from yeast cells to germ tubes. During initial invasion, when germ tubes extended into elongated hyphae, we observed further strong expression of hyphae-associated genes. For example, *ALS3*, a gene encoding the first discovered *C. albicans* invasin involved in epithelial up-take of hyphae (induced endocytosis) (Phan *et al.*, 2007), was strongly up-regulated during the first two phases of infection. The dissemination phase, however, was characterised by the up-regulation of genes involved in the response to several stresses such as carbon starvation, limited nitrogen and phosphate sources and nitrosative stress. These data show that the fungus is challenged with strong environmental changes during invasion and damage of human oral epithelial host cells (Zakikhany *et al.*, 2007). A previously unknown function gene was up-regulated in these experimental oral infections as well as in oral samples isolated from patients suffering from oral candidosis. A deletion mutant lacking this gene showed an interesting phenotype: after initial contact with human host cells it formed germ tubes and was able to invade the superfical layers of RHE. Inside host cells however, the mutant did not extent germ tubes into hyphae and switched back to yeast-like growth. These yeast cells remained trapped inside the superfical RHE layers and did not disseminate into the deeper tissue parts. Therefore, the gene was called *EED1* (**E**pithelial **E**scape and **D**issemination 1) (Zakikhany et al., 2007). Further experiments have shown that *Eed1* is an unique key regulator of hyphal extension in *C. albicans* and plays an essential role in the maintenance of filamentous growth and virulence in several infection models (Martin *et al.*, revised).

This work is financed in part by the Deutsche Forschungsgemeinschaft (DFG) priority program 1160 (Hu528/10).

References

Schaller M et al. (2006) *Nature Protocols* 6, 2767–2773.

Zakikhany K et al. (2007) *Cell Microbiol* 9, 2938–2954.

Phan QT et al. (2007) *PLoS Biol* 5, e64.

3 Infection-associated Genes of *Candida albicans*

Group Leaders: François Mayer, Duncan Wilson, Bernhard Hube

Infection-associated genes

Transcriptional profiling is a powerful tool for dissecting the molecular mechanisms that an organism employs in response to specific environments. We have taken advantage of this technology to unravel the biology underlying *Candida albicans* infections; by analysing the transcriptome of this pathogen during various types of infection, we have begun to dissect which genes, regulons and biological processes are relevant during the infection process. Gene expression data has provided evidence about the types of microenvironments that the fungus encounters during infection of the host. For example, during liver infection, key enzymes of glycolysis, acetyl-CoA biosynthesis and the TCA cycle were up-regulated, suggesting that *C. albicans* is able to utilise sugars during infection of this organ. On the other hand, within oral tissue, it appears that glucose is not available, as indicated by the up-regulation of components of gluconeogenesis and the glyoxylate cycle. Although capable of providing such detailed evidence about the pathogen's behaviour *in vivo*, a major problem still exists in the interpretation of such datasets: usually, a large proportion of significantly regulated genes are of unknown function. This problem is compounded by the fact that most annotations of the *C. albicans* genome have been achieved based on homology with the non-pathogenic yeast, *Saccharomyces cerevisiae*. We therefore hypothesised that genes of unknown function, which are specifically expressed during infection ("infection-associated genes") are strong virulence factor candidates. Therefore, in this project, "infection-associated genes" were scrutinised *in silico* using bioinformatics tools to search for functional domains, localisation signals and relatedness to other species. Based on these analyses, a set of 50 genes with a high likelihood of involvement in host-pathogen interactions were selected for further investigation. In order to gather detailed information about

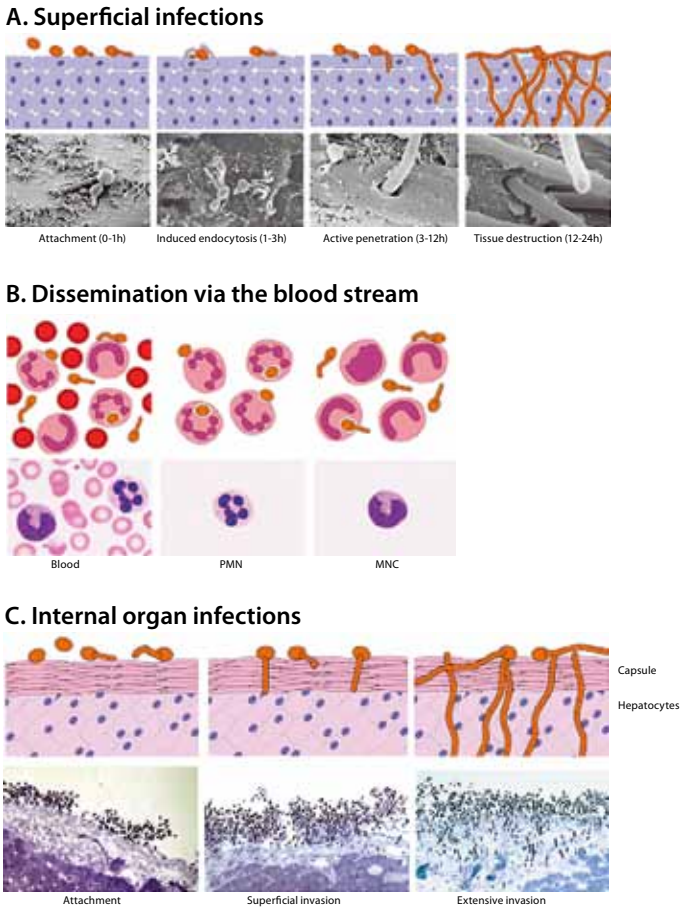

Figure 3
Distinct stages of candidosis. *Candida albicans* can cause superficial infections of mucosal surfaces (A); but this fungus can also gain access to the bloodstream (B) and from there disseminate to and infect internal organs such as the liver (C). For infection of solid organs/tissue, the fungus first adheres to host cells before invading and causing damage. In the blood stream the fungus must deal with host immune cells such as polymorphonuclear (PMN) and mononuclear (MNC) cells.

the roles of these genes during host-pathogen interactions, a number of approaches were taken. Primarily, expression of infection associated genes under different conditions was analysed in detail using GeneSpring software. Using this technique, we were able to define the transcriptional modularity of *C. albicans* during clinically relevant events and characterise gene sets that are co-expressed under specific subsets of conditions. By combining information derived from these regulons with additional functional genomic data, we were able to begin to assign novel functional annotations for uncharacterised genes. For further characterisation, mutants were generated with specific inactivations of infection-associated genes. Because *C. albicans* is diploid and lacks a fully defined sexual cycle, classical genetics cannot be performed with this organism and advanced molecular biology approaches are required for genetic characterisation. The disruption strategy we employ is based on a rapid PCR method (Gola *et al.*, 2003) using strain BWP17, which is auxotrophic for arginine, histidine and uridine. First, ARG4- and HIS1-disruption cassettes were generated using primers containing approximately 100 bp homology to the immediate upstream and downstream sequences of the relevant target gene. The ARG-disruption cassettes were then used in a primary transformation event to disrupt the first copy of the relevant gene of interest by homologous recombination. Heterozygotes were then transformed in a second transformation process with the HIS-disruption cassettes. Correct deletion of both alleles of the target gene was verified by diagnostic PCR and Southern blot analysis. In the final step, the remaining uridine auxotrophy of the homozygous mutant is used to restore a wild type copy of the gene of interest, thus satisfying Molecular Koch's postulates.

Unlike certain bacterial pathogens, where production of a single toxin is sufficient for disease manifestation, the pathogenicity of

References

Gola S et al. (2003) *Yeast* 20, 1339–1347.

Zakikhany et al. (2007) *Cell Microbiol* 9, 2938–2954.

Fradin et al. (2005) *Mol Microbiol* 56, 397–415.

Thewes et al. (2007) *Mol Microbiol* 63, 1606–1628.

References
Ramirez MA and Lorenz MC. (2007) *Eukaryot Cell* 6, 280–290.

C. albicans relies on a range of virulence factors and fitness attributes such as the yeast-to-hyphal transition, expression of adhesins, expression of invasins, secretion of hydrolytic enzymes, cell wall biogenesis, essential nutrient acquisition, together with metabolic flexibility and adaptation to stress. For these reasons, our set of infection associated gene mutants were first tested *in vitro* for their ability to form hyphae, invade agar, utilise alternative nutrient sources and respond appropriately to various stresses. Next, mutants were assayed in defined infection models for their ability to adhere to and damage various types of human cells. By analysing the behaviour of our mutants in both classical *in vitro* conditions and in defined infection models we are able to both assign biological function whilst simultaneously determining the role of the gene product during infection. We have begun to unravel the biological function of some of these genes and define their role during infection. In addition, we have developed a novel *in vitro* model for analysing a key step during systemic candidosis.

BIS1 mediates stress adaptation and virulence

This gene was chosen for further investigation as it featured a strong upregulation during liver infection, interaction with neutrophils and macrophages and under oxidative stress. *In silico* analysis revealed that this uncharacterised gene encodes a putative small heat shock protein (sHSP). Based on its strong upregulation in the blood infection model and the function as a possible sHSP, the gene was named *BIS1* (**b**lood **i**nduced **s**tress protein 1). A primary screen for growth of a *bis1*Δ knock out mutant revealed that this gene is required for tolerance against specific stresses including thermal, oxidative, DTT and ethanol stress. Interestingly, osmotic stress bypassed *BIS1* dependent thermal tolerance. The *bis1*Δ mutant displayed reduced survival during interaction with neutrophils. Moreover, *BIS1* was required for full virulence in an *in ovo* infection model and demonstrated to be important for damage of both epithelial and endothelial cells. Taken together these results provide novel links between stress adaptation and fungal pathogenicity.

OCS2 encodes a predicted membrane protein required for invasive hyphal development and oral epithelial tissue damage

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

An *in vitro* model of the circulatory system

The most serious form of candidosis occurs when this fungus gains access to the blood system. However, before infection of internal organs can occur, *C. albicans* must first adhere to and then traverse the endothelial lining of blood vessels under conditions of physiological pressure. We therefore have developed an *in vitro* model for studying this key step. We discovered that a distinct stage in the yeast to hypha transition, mediated by the G1 cyclin Hgc1, is critical for endothelial adhesion under such conditions of physiological flow.

This work is financed in part by the International Leibniz Research School (ILRS) and the Marie Curie Intra-European Fellowship project “Interaction of fungal pathogens with host cells: a post-genomic approach”.

4 Localized virulence associated gene expression pattern of *Candida albicans*

Group Leaders: Anja Lüttich, Sascha Brunke, Ilse Jacobsen, Bernhard Hube

Candida albicans is both a successful commensal and a pathogen of humans that can infect a

broad range of body sites. The transition from commensalism to parasitism requires a susceptible host, but it is also an active process. Gene expression of *C. albicans* is regulated by an interplay between host and pathogen and, most likely, distinct transcriptional programs. Factors which determine the virulence of *C. albicans* are e. g. the expression of metabolic enzymes, mechanisms for the uptake of amino acids and dimorphism (Barelle *et al.*, 2006, Lorenz *et al.*, 2001, Lo *et al.*, 1997). However, there are many more factors which remain to be investigated or even discovered. Evolutionary adaptation of pathogens during interaction with host cells or within host environments occurs through the accumulation of mutations that improve the reproductive success of an organism. Studies on *C. albicans* resistance to antifungals have shown that the fungus can very rapidly (within weeks) adapt genetically to changing microenvironments (Cowen *et al.*, 2000). Serial passage experiments display a form of experimental evolution to monitor molecular and phenotypic evolution in real time (Ebert *et al.*,1998).

In this project we focus on two related topics: (1) Identification of virulence associated gene expression pattern and virulence associated genes during interaction with host cells using infection models, laser capture technologies and microarrays; and (2) identification of virulence associated gene expression pattern and virulence associated genes using infection models and microevolution experiments.

Identification of virulence associated gene expression pattern and virulence associated genes using laser capture technologies

The capability to become a pathogen or the success of a pathogen relies on a rapid transcriptional response to the constantly changing microenvironments on or within the host. Therefore, *C. albicans* possesses different transcription programs and consequently expresses several virulence associated genes during the different stages of infection (Wilson *et al.*, 2009). Elucidating the genes expressed during infection may help to identify new putative drug targets or diagnostic markers.

The project aims to overcome one key limitation of all current available transcriptional technologies for *in vitro* and *in vivo* transcriptional profiling: that they are based on the transcript profile of populations of cells. The microdissection technology allows monitoring of local gene expression pattern (single cell profiling) during interaction with host cells and, consequently, may help to detect genes specifically expressed during distinct stages of *Candida albicans*-host interactions on a cellular level. The laser capture process does not alter or damage the morphology and chemistry of the sample collected, nor of the surrounding cells. Thus the isolated cells can be used directly for RNA isolation. We started to establish an RNA isolation protocol using very low numbers of fungal cells followed by RNA-amplification. Currently, we are able to isolate RNA from a minimum of 1000 *Candida* cells, and to amplify these low RNA amounts to obtain sufficient starting material for microarray analysis. Furthermore, we aim to establish suitable infection models and sample collection protocols for our *in vitro* and *in vivo* microdissection technologies.

Identification of virulence associated gene expression pattern and virulence associated genes using microevolution experiments

During the infection process, *C. albicans* has the potential to adapt to different host niches and to survive the attack of phagocytes, such as macrophages (Barelle *et al.*, 2006; Enjalbert *et al.*, 2007; Hube *et al.*, 2004).

We use an experimental *in vitro* microevolution approach to identify factors and activities necessary for survival within, and escape from, macrophages. The advantage of *in vitro* evolution compared to analysing *in vivo* adaptations in patient samples is that experiments are subject to strict control of conditions (e. g. population size, strength of selection) and are easy to repeat and to reproduce. To monitor microevolutionary changes during interaction with host cells, we established a protocol for *C. albicans*-macrophage interactions. From a collection of existing hyphal-deficient mutants, we identified one strain which cannot escape from macrophages, while the wild type can

References

Barelle CJ et al. (2006) *Cell Microbiol* 8, 961–971.

Cheng S et al. (2007) *Cell Microbiol* 9, 492–501.

Cowen LE et al. (2000) *J Bacteriol* 182, 1515–1522.

Ebert D (1998) *Science* 282, 1432–1435.

Enjalbert B et al. (2007) *Infect Immun*, 2143–2151.

Hube B (2004) *Curr Opin Microbiol* 7, 336–341.

Lo HJ et al. (1997) *Cell* 90, 939–949.

Lockhart SR et al. (1995) *J Clin Microbiol* 33, 1501–1509.

Lorenz MC, Fink GR (2001) *Nature* 412, 83–86.

Mavor AL et al. (2005) *Curr Drug Targets* 6, 863–874.

Shin HJ et al. (2004) *J Clin Microbiol* 42, 4025–4031.

Wilson D et al. (2009) *FEMS Yeast Res* 9, 688–700.

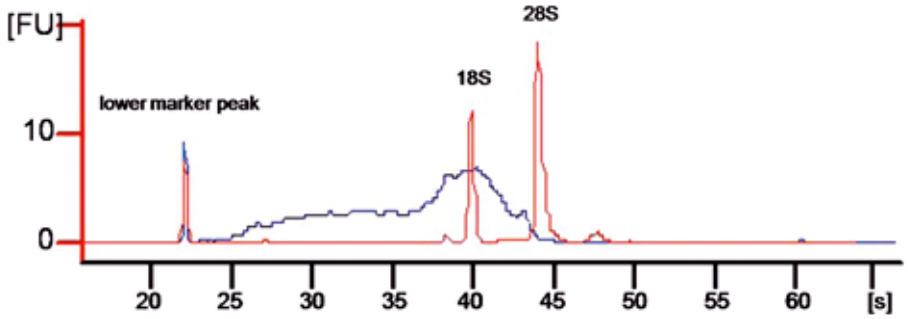

Figure 4
Quality of *Candida albicans* total RNA measured by BioAnalyzer.
(Red: high quality / Blue: low quality RNA sample / FU: fluorescence / s: seconds)

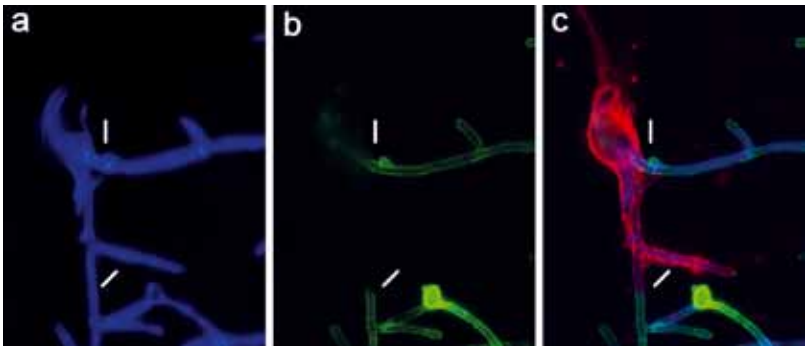

Figure 5
Morphology of *Candida albicans* wild-type cells in macrophages (red)
a) Calcofluor-white staining (extracellular + internalized cell),
b) Concanavalin-A staining (extracellular cell),
c) merged; white lines show piercing of the macrophage by *C. albicans*

escape and kill macrophages. Eight mutants lacking regulators of hyphal formation were tested for their behavior during co-incubation with macrophages as compared to wild type cells. Four hours after infection, more than half of wild type cells escaped from the macrophages. In contrast, none of the tested mutants achieved such high escape rates. Since the *efg1*Δ/*cph1*Δ mutant is unable to produce hyphae under almost all tested conditions and unable to escape from macrophages, we chose this mutant for our microevolution experiment and investigated the adaptation of this mutant within macrophages during a series of passages.

After several passages, *efg1*Δ/*cph1*Δ exhibited a dramatic phenotypic alteration and started to form filaments. These filaments enabled the evolved strain to escape from macrophages. Interestingly, this strain also formed filaments under several other tested hyphal inducing conditions, in contrast to the original *efg1*Δ/*cph1*Δ mutant. This phenotype was stable, indicating that a microevolutionary event has bypassed the mutations of the two key hyphal regulators *CPH1* and *EFG1*. We have begun to elucidate the genetically stable modifications of the evolved strain by using microarrays and karyotype analysis.

Similarly, we are preparing to conduct an *in vivo* microevolution experiment to identify factors necessary for survival, immune evasion and/or tissue destruction in the host. To date, only few studies have performed such microevolution experiments *in vivo*. The mechanisms and rates by which phenotypic and genotypic variation are generated during growth in the host are not well understood.

In the past, different groups showed microevolution in a series of *C. albicans* isolates from patients using pulsed-field gel electrophoresis, DNA fingerprinting methods and restriction endonuclease analysis of genomic DNA (Lockhart *et al.*, 1995; Shin *et al.*, 2004).

Others have found that *C. albicans* can become attenuated by continued in vivo passages through murine spleens (Cheng *et al.*, 2007). Since the kidneys are main target organs during invasive candidosis (Mavor *et al.*, 2005), and *C. albicans* can persist in these organs despite a pronounced immune defence, we focussed on isolates from this organ for our microevolution experiments.

This project is financed in part by the Jena School for Microbial Communication (JSMC).

5 Infection-associated genes and molecular biology of pigment production of *Candida glabrata*

Group Leaders: Katja Seider, Sascha Brunke, Bernhard Hube

The principal aim of this projects is to study the increasingly important human fungal pathogen, *Candida glabrata*, to better understand the molecular mechanisms of its pathogenicity.

C. glabrata is part of the typical microflora on human mucosal surfaces. Like *C. albicans*, it is considered to be a normally benign human commensal, but can turn into a successful opportunistic pathogen, which causes up to 20 % of mycoses in the clinical setting – by now it has become the second most frequent cause for candidemia after *C. albicans*. The high risk of *C. glabrata* infections arises from the intrinsically high resistance to widely used antifungal drugs. During antimycotic treatment, it can thereby outcompete and replace other *Candida* species in the patient. Indeed, treatment efficacy of *C. glabrata* infections has not improved during the last decade.

Besides its relative resistance to antifungal drugs, only few virulence attributes, such as adhesion factors and phenotypic switching, have been described for this fungus, and infection mechanisms remain poorly understood. Furthermore, key aspects in *C. albicans* tissue invasion and persistence, most notably filamentation by hyphal formation and secretion of proteases, have no parallels in *C. glabrata*. Nevertheless, despite its lack of filaments, *C. glabrata* is still invasive *in vivo* and can cause severe infections, mainly in immunocompromised patients.

Candida glabrata interactions with human cells of the innate immunity

Although we still do not know how *C. glabrata* disseminates in immunocompromised patients, there are ways – either through direct penetration from epithelial tissues or due to damage of barriers in the body caused by surgery, polytrauma or drug treatment – to enter the bloodstream, to distribute and to infect other organs. In consequence *C. glabrata* must have developed strategies to counteract or bypass mammalian host defence systems, thereby enabling the fungus to cause systemic disease.

Interestingly, in a systemic mouse model, which was established in our group, colonisation within organs causes very little inflammation. Nevertheless, yeast cells can be re-isolated over a surprisingly long period from infected animals. This suggests that immune evasion strategies might play a key role during infection with *C. glabrata*. Additionally, it has been proposed that *C. glabrata* can survive the attack of phagocytes and can even replicate within macrophages after engulfment (Kaur *et al.* 2007; Roetzer *et al.* 2009).

Due to these observations we aim to dissect the interaction between *C. glabrata* and human monocyte-derived macrophages (MDM). We started to look at the general phagocytic pathway of *C. glabrata* in primary macrophages and confirmed that *C. glabrata* survives and replicates in a membrane-surrounded phagosome. Our data equally suggest that the normal events of phagosome maturation, which normally lead to the killing of phagocytosed microbes, are actively disrupted by this pathogen. In particular, phagosomal acidification and the recruitment of the lysosomal enzyme cathepsin D is blocked by viable, but not killed fungi. Genome-wide transcriptional profiles of *C. glabrata* cells exposed to media with different pH values or of phagocytosed cells support

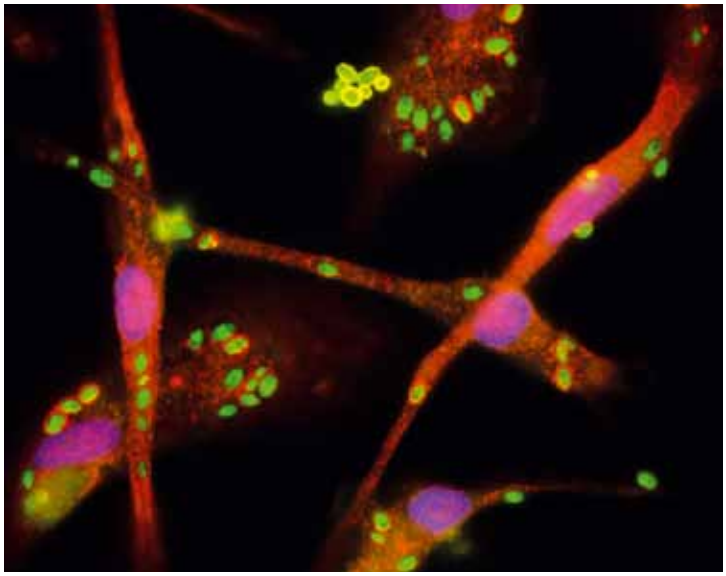

Figure 6
C. glabrata (green) phagocytosed by human monocyte-derived macrophages (red).

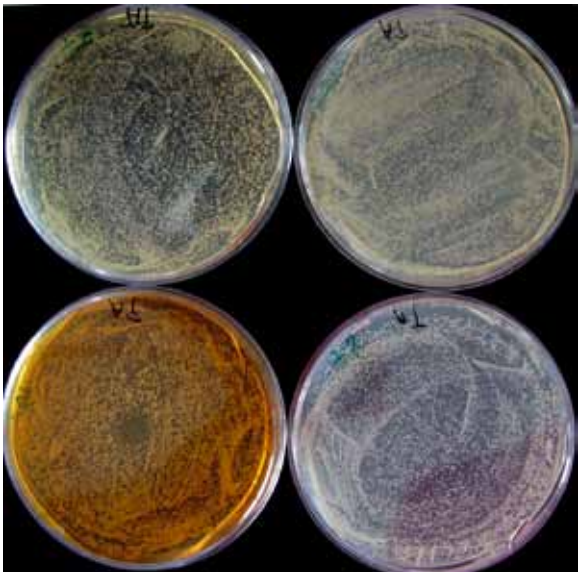

Figure 7
C. glabrata deletion mutants of Ehrlich pathway genes. Top: wild type and *aro9Δ* mutant; bottom: *aro10Δ* and *aro8Δ* mutants. Deletion mutants lacking the *ARO8* gene produce no pigments, while those lacking *ARO10* are hyperpigmented.

References
Kaur et al. (2007) *PNAS* 104, 7628–3763.

Roetzer et al. (2009) *Cell Microbiol* 12, 199–216.

the view that *C. glabrata* cells within MDMs were not exposed to an acidic pH. Despite significant intracellular proliferation of fungal cells, apoptosis or damage of macrophages was not apparent and reactive oxygen species, produced by macrophages, were either scavenged by fungal acitivites or their production inhibited by the fungus. In addition, levels of the pro-inflammatory cytokines IL-1β, IL-6, IL-8, IFNγ and TNF-α compared to uninfected controls were only marginally increased. In conclusion, it is possible that *C. glabrata* evades attacks by other immune cells, such as neutrophil granulocytes, by hiding within macrophages.

A second part of this project deals with the identification of genes and activities of *C. glabrata* necessary to avoid killing by macrophages as well as the elucidation of fungal strategies to suppress an inflammatory response. To this end, we screened a library of *C. glabrata* mutants for their ability to survive inside the phagocyte. Mutants that do show an altered survival rate, will be further characterized in a number of *in vitro*, *ex vivo* as well as *in vivo* models to investigate the physiological relevance of the lacking gene.

Molecular biology of pigment production of *Candida glabrata*

Pigment production is a common phenomenon in fungi, and many important fungal pathogens of humans are pigmented. *Aspergillus* species, *Cryptococcus neoformans*, and *Histoplasma capsulatum*, to name just a few, all produce dark brown or black melanin. Its protective functions against oxidants, hydrolytic enzymes and other detrimental environmental factors play important roles for these fungi in surviving the human immune response.

The pathogenic yeast *C. glabrata* produces a different reddish-brown pigment, consisting of a complex mixture of indolic compounds. It is induced by the presence of tryptophan,

and has been found in several other fungi like *C. neoformans* or *M. furfur*. We are interested in the possible biological roles of this pigment and the biosynthetic pathway of its formation.

Pigment synthesis in *C. glabrata* seems to be regulated by a multitude of factors, with the presence of tryptophan being the only absolute prerequisite. Synthesis is influenced for example by the presence of different nitrogen sources, like ammonium or other amino acids. Depending on the nitrogen source, pigmentation can be delayed or inhibited completely. Also, cell density has a strong effect on synthesis, with high cell numbers suppressing pigment production. Furthermore, we were able to show that cAMP is involved in at least some of these regulatory processes. Pigmentation protects *C. glabrata* from the detrimental effects of UV light and oxidants like hydrogen peroxide, and the pigment reduces the ability of neutrophils to damage *C. glabrata* yeasts. This implies a role of pigmentation in pathogenesis, for example by supporting the fungus to survive encounters with immune effector cells. In these respects, the properties of the pigment are similar to the established functions of fungal melanins, commonly called "fungal shield". Furthermore, we could demonstrate that culture supernatant containing soluble pigment is able to suppress the yeast-to-hypha transition of *C. albicans*, which plays a significant role in the pathogenesis of this fungus. While this cross-species effect may in part be explained by the synthesis of tryptophol as a by-product of the pigment, further pigment constituents are involved.

In an effort to elucidate the synthesis pathway of the pigment, we used a transposon insertion library of random mutants and transcriptional profiling of pigment producing *C. glabrata* cells. With these methods, we were able to localize the central biochemical processes of pigment synthesis to the Ehrlich pathway, the catabolic reactions necessary for degradation of aromatic amino acids. In fact, deletion of a single gene, the aromatic aminotransferase *ARO8*, led to a nearly complete loss of pigmentation. When we expressed this gene heterologously in *E. coli*, we found that the encoded enzyme alone is sufficient for *in vitro* synthesis of the pigment.

Thus, pigmentation in *C. glabrata* is a by-product of a single enzymatic step involved in the catabolism of tryptophan, but has a multitude of possible biological functions for this fungus.

This work is financed in part by the Federal Ministry of Education and Research (BMBF) and the EU ERA-NET PathoGenoMics Programm "Genomic Approaches to unravel the molecular basis of pathogenicity in the Human Fungal Pathogen *C. glabrata* – FunPathCaGla".

6 Iron sources during oral infections

Group Leaders: Ricardo Sergio Couto de Almeida, Bernhard Hube

Iron is an essential element for the vast majority of all organisms from microbes to multicellular animals. Using high affinity iron-binding molecules, higher organisms can keep compartments almost free of iron causing a natural resistance to infections which can be described as "nutritional immunity". Therefore, pathogenic microbes are forced to utilize iron from host molecules during infection. For example, within the oral cavity, extracellular iron is mostly bound to lactoferrin found in saliva and intracellular iron is stored by ferritin.

Ferritin is the main intracellular storage protein for iron containing thirteen percent of the total human body iron (66 % are bound in hemoglobin). Ferritin consists of a 24-subunit protein shell of approximately 500 kDa. One ferritin molecule can contain up to 4,500 hydrolyzed Fe^{3+} ions. The quaternary structure of ferritin is dissociated at acidic pH. Under iron-limiting conditions, cytosolic ferritin is autophagocytosed and subsequently degraded within the acidic lysosomes and the iron is allocated to the cell. Outside of lysosomes, ferritin is an extremly robust and stable protein which seems to be resistant to all known mi-

References
Hoyer LL (2001) *Trends Microbiol* 9, 176–180.

Phan QT et al. (2007) *PLoS Biol* 5, e64.

Sheppard DC et al. (2004) *J Biol Chem* 279, 30480–30489

crobial activities. In fact, the only microorganism that has so far been shown experimentally to exploit holoferritin as an iron source during interaction with host cells is *Neisseria meningitidis*.

Candida albicans is a polymorphic yeast which belongs to the normal microbial flora of human beings. The fungus lives as a harmless commensal on mucosal surfaces in healthy individuals but can cause several types of infections in predisposed patients, ranging from superficial to life threatening disease. To acquire iron, *C. albicans* possesses three known iron up-take pathways:
(1) Up-take and usage of iron from hemoglobin. This pathway is composed of a hemoglobin receptor (Rbt5) and a hem oxygenase (Hmx1) which degrades hemoglobin to release the iron.
(2) The siderophore receptor Sit1 can mediate up-take of a range of siderophores (iron chelators produced by microorganisms) from other organisms and other iron complexes.
(3) To use free iron from the environment or iron from transferrin and maybe other so far unknown iron sources, *C. albicans* uses the reductive up-take system. This system is located in the plasma membrane and consists of three activities. Firstly, insoluble extracellular ferric (Fe^{3+}) chelates can be reduced into soluble Fe^{2+} ions by reductases. Since reduced ferrous iron generated by surface reductase can be toxic by spontaneous generation of free radicals, the Fe^{2+} ion is subsequently oxidized to Fe^{3+} and transported into the cell by a protein complex consisting of a multicopper oxidase and an iron permease.

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

By using an iron chelator to remove free iron from the media we could show that *C. albicans* can use ferritin as the sole source of iron *in vitro*. This is mediated by acidification of the medium and requires the reductive pathway.

Because *C. albicans* can use ferritin as a sole source of iron, we investigated whether *C. albicans* can bind ferritin on its surface. For this *C.albicans* wild type and mutant cells lacking key genes required for hyphal formation were incubated under hyphal-inducing conditions. Using fluorescent stained anti-ferritin antibodies we could show that hyphal, but not yeast cells of *C. albicans* can bind ferritin.

Transcriptional profiling of *C. albicans* cells binding ferritin identified putative genes necessary for ferritin binding. One of them ALS3 (Agglutinin-Like Sequence) encodes a hyphal-specific cell wall protein which belongs to a family of adhesins (*ALS* family) (Hoyer, 2001) and plays a crucial role in epithelial and endothelial adhesion and invasion (Phan *et al.*, 2007). Deletion of *ALS3* precludes ferritin binding which suggests that *ALS3* plays a crucial role in ferritin binding and may in fact be the hyphal-specific ferritin receptor.

To elucidate whether Als3 itself can bind ferritin without an additional *C. albicans* surface factor, we tested the ferritin binding capacity of a strain of *S. cerevisiae* that expressed *C. albicans ALS3* (Sheppard *et al.*, 2004). Because *ALS3* is a member of a large gene family encoding similar proteins, we also analyzed two additional *S. cerevisiae* strains that expressed *ALS1* or *ALS5*, two closely related *ALS* genes. The strain that expressed *ALS3* strongly bound ferritin, whereas the strains that expressed *ALS1* or *ALS5* did not. This result supports the hypothesis that Als3 is a ferritin receptor.

Next we investigated whether ferritin binding via Als3 occurs when *C. albicans* interacts with host cells. For this oral epithelial cells were loaded with iron and then incubated with wild-type *C. albicans*, the *als*3Δ mutant, or the *als3*Δ (*ALS3*) re-integrant strain. We could show that hyphae of wild type and *als3*Δ (*ALS3*) re-integrant strains invaded the epithelial cells and were surrounded by ferritin. Very little ferritin accumulated around wild type hyphae that had not invaded the epithelial cells. In contrast, the few hyphae of the

*als3*Δ mutant that invaded the epithelial cells displayed no accumulation of ferritin. These results indicate that *C. albicans* hyphae bind to ferritin in an Als3-dependent manner while invading epithelial cells.

In addition to that the *als3*Δ mutant was unable to damage epithelial cells in vitro.

Therefore we can proclaim that Als3 has an additional function. Beside its known adhesion properties and function as invasin we made the intriguing observation that Als3 plays a role in iron acquisition by binding to host ferritin, indicating that this single member of a protein family has multiple virulence-associated functions.

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7 Proteases as virulence factors

Group Leaders: Lydia Schild, Antje Heyken, Bernhard Hube

Proteolytic enzymes fulfil multiple cellular functions such as protein degradation, activation of protein precursors and digestion of nutrients. Moreover, in pathogenic microorganisms secreted proteases can act as important virulence factors. They enable pathogens to invade host tissues or to evade the host immune system. We work on the family of secreted aspartic proteases (Saps), one major group of virulence factors which contribute to pathogenicity of *Candida albicans*. Among the ten described Sap proteases, Sap9 and Sap10 take an exceptional position (Albrecht *et al.*, 2006).

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

Sap9 and Sap10 are highly glycosylated proteases, which are GPI-anchored on the cell surface

To determine whether Sap9 and Sap10 are in fact GPI proteins, we used a series of constructs to express native and C-terminal truncated versions of Sap9 and Sap10 in the yeast *Pichia pastoris*. While expression of native Sap9 and Sap10 prevented secretion of these proteases, secretion was observed when parts of the C-terminal sequences of the GPI anchor consensus sequence were deleted.

For determination of cellular localization of the two proteases, we constructed Sap9- and Sap10-Gfp fusion proteins. Immunoelectron microscopy using an anti-Gfp antibody revealed that both fusion proteins are located on the cell surface of *C. albicans*; Sap9-Gfp was predominantly located in cell membranes, whereas Sap10-Gfp was located in both the cell membrane and the cell wall.

In contrast to other members of the Sap family, which possess a maximum of one potential *N*-glycosylation site within the sequence of the mature enzyme, Sap9 and Sap10 contain, respectively, five and eight such sites. Treatment of Sap9 and Sap10 with *N*-glycosidase F caused a clear band shift for both proteases, suggesting that Sap9 and Sap10 are *N*-glycosylated.

Sap9 and Sap10 function in cell surface integrity

To determine the possible functions of the two proteases, we constructed *sap9*Δ and *sap10*Δ single mutants and a *sap9*Δ/*sap10*Δ double mutant. All mutant strains grew normally on media containing different carbon sources, at

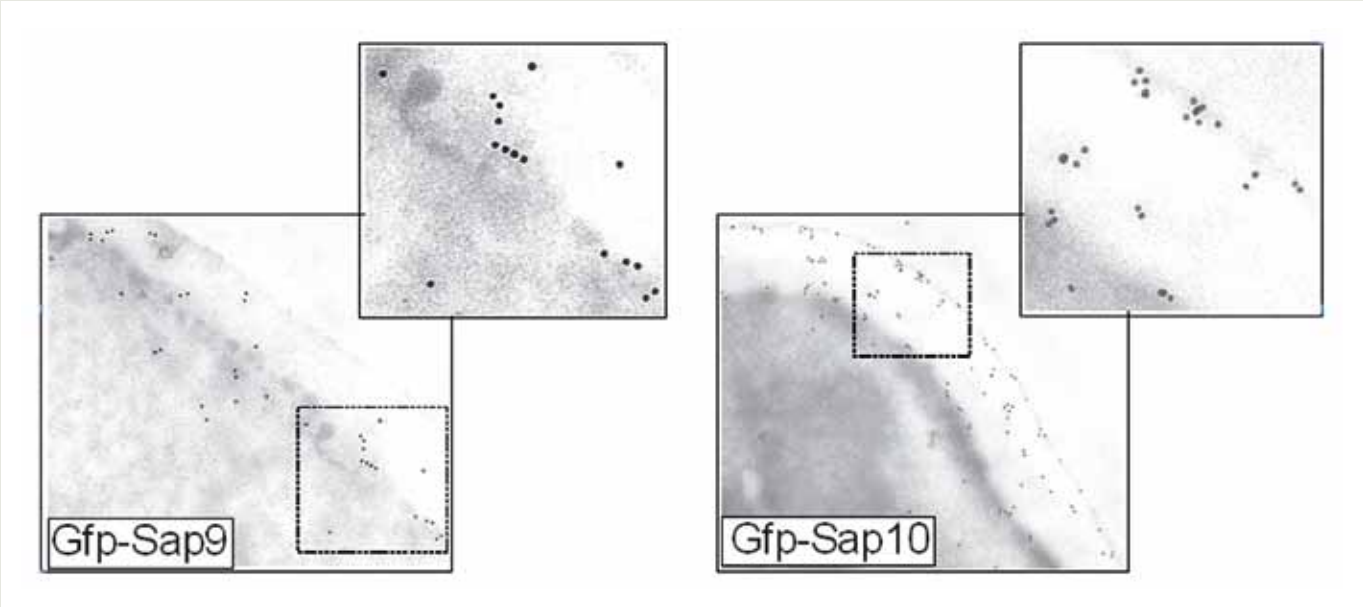

Figure 7
Localization of Sap9- and Sap10-Gfp fusion proteins as shown by immunoelectron microscopy and gold-labeled anti-Gfp antibodies. Sap9-Gfp is predominantly located in cell membranes, whereas Sap10-Gfp is located in both the cell membrane and the cell wall.

References

Eisenhaber B et al. (2004) *J Mol Biol* 337, 243–253.

Hornbach A et al. (2009) *Infect Immun* 77, 5216–5224.

Naglik J R et al. (2003) *Microbiol Mol Biol Rev* 67, 400–428.

Meiller TF et al. (2009) *PLoS One* 4, e5039.

Gropp K et al. (2009) *Mol Immunol* 47, 465–475.

Komano H et al. (1999) *J Biol Chem* 274, 24431–24437.

Olsen V et al. (1998) *Biochem* 37, 2768–2777.

Rockwell NC et al. (1998) *Biochem* 37, 3386–3391.

Albrecht A et al. (2006) *J Biol Chem* 281, 688–694.

different pH values, on hyphal inducing media or when incubated at different temperatures. However, components that directly or indirectly target the fungal cell surface such as hygromycin B, amorolfine, calcofluor, Congo red, and itraconazole caused significant growth defects for all mutants. Furthermore, all three mutants exhibited an abnormal budding phenotype, as daughter cells did not separate from mother cells and remained associated in cell chains. Because it is known that blockage of protein activities involved in cell membrane or cell wall maintenance can be compensated for by production of alternative structural elements, we quantified the protein and chitin levels in the cell walls of the mutants. Chitin levels of the *sap9*Δ mutant were significantly increased whereas the *sap10*Δ mutant showed increased levels of both protein and chitin as compared with the parental strain.

Role of Sap9 and Sap10 in *C. albicans* host interaction

To investigate whether dysfunctions resulting from *SAP9* or *SAP10* deletion influenced virulence of *C. albicans*, we and others investigated the interaction of *sap9*Δ and *sap10*Δ mutants with epithelial cells and immune cells.

Both *sap9*Δ and *sap10*Δ mutants had a significantly reduced ability to invade and damage epithelial cells in a model of oral infection based on RHE (Reconstituted human epithelium). In the case of *sap10*Δ, this attenuation is likely to be caused by a reduced ability to adhere to epithelial cells. However, since adhesion of the *sap9*Δ mutant was increased, properties other than adhesion must be responsible for the decreased epithelial cell damage of *sap9*Δ. Summarized, activity of Sap9 and Sap10 is necessary for wild type adhesion properties and invasion and cell damage of oral epithelial cells.

Since the cell surface is the major contact point between *C. albicans* and the immune system, we expect that Sap9 and Sap10 activities influence *C. albicans* immune cell interactions and immune evasion. Unmasking of fungal cell wall components due to lack of *SAP9* or *SAP10* could cause an enhanced recognition and ingestion of *C. albicans* by phagocytes. We have therefore begun to analyse the interaction of *sap9*Δ and *sap10*Δ mutants with human macrophages.

During interaction with human neutrophils, Sap9 plays an important role for the recognition and killing of *C. albicans*. *SAP9* is required for the induction of neutrophil chemotaxis toward *C. albicans* filaments. Deletion of *SAP9* leads to a mitigated release of reactive oxygen intermediates (ROI) by human neutrophils and increased survival of the fungus (Hornbach *et al.*, 2009).

In addition to direct interaction with immune cells, *C. albicans* proteases may evade host defenses by directly degrading molecules such as antimicrobial peptides and complement factors (Naglik *et al.*, 2003). Sap9 has been shown to cleave and inactivate the antimicrobial peptide histatin 5, which is secreted by epithelial cells (Meiller et al., 2009). In contrast to Sap1, Sap2 and Sap3, Sap9 seems to be dispensable for immune evasion by degradation of host complement components (Gropp *et al.*, 2009).

Proteolytic properties and substrates of Sap9 and Sap10

To characterize attributes of Sap9 and Sap10 proteolytic cleavage, we monitored specific activity of the proteases by quantifying the cleavage of a fluorogenic peptide. Both proteases showed highest activity in a pH-range of 6 to 7 and were only incompletely inhibited by the aspartic protease inhibitor Pepstatin A.

Sap9 and Sap10 cleavage preferences were determined using proteases heterologously expressed in *Pichia pastoris* in cleavage assays with synthetic peptides. Peptides containing basic or dibasic amino acid motifs were digested by Sap9 or Sap10 or by both proteases. Digests were similar to the activity of *S. cerevisiae* yapsins or Kex2 regulatory proteases with hydrolysis at KR, KK, or single Lys (K) sites. Sap9 and Sap10 preferred cleavage after dibasic (KR, KK) or monobasic (Lys, Arg) residues, similar to yapsin 1 and 2, whereas the Kex2 protease cleaves only C terminally to clusters of dibasic residues (Komano *et al.*, 1999; Olsen et al., 1998; Rockwell *et al.*, 1998). In addition, Sap10 digested at sites previously unknown for yapsin-like aspartic proteases (between Phe-Ser and His-Asn). Further studies showed that dibasic residues are common but not exclusive motifs in Sap9 and Sap10-digested peptides and amino acids surrounding dibasic residues are important determinants of cleavage preference.

Because Sap9 and Sap10 are exposed to the extracellular space, but attached to the cell membrane or cell wall, we concluded that proteolysis by Sap9 and Sap10 must mainly take place on the cell surface and that putative substrates of these proteases may be of fungal origin. To identify possible target proteins of Sap9 and Sap10, we digested isolated cell walls with recombinant Saps. Using LC/MS/MS we detected the release of seven GPI-anchored proteins following protease digestion: Cht2, Ywp1, Als2, Rbt5, Ecm33, Pga4 and Pga29. These potential target proteins have distinct functions from cell wall remodelling to host cell adhesion. For one candidate, Cht2, a cell-associated chitinase, functional assays revealed that protein activity is reduced in the absence of Sap9 and Sap10.

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8 Interaction of *Candida albicans* with neutrophils

Group Leaders: Antje Heyken, Pedro Miramón, Ines Leonhardt, Bernhard Hube

Granulocytes govern *Candida albicans* transcriptional response

The gene expression patterns of *Candida albicans* cells exposed to whole blood or to neutrophils are similar. Thus, neutrophils govern

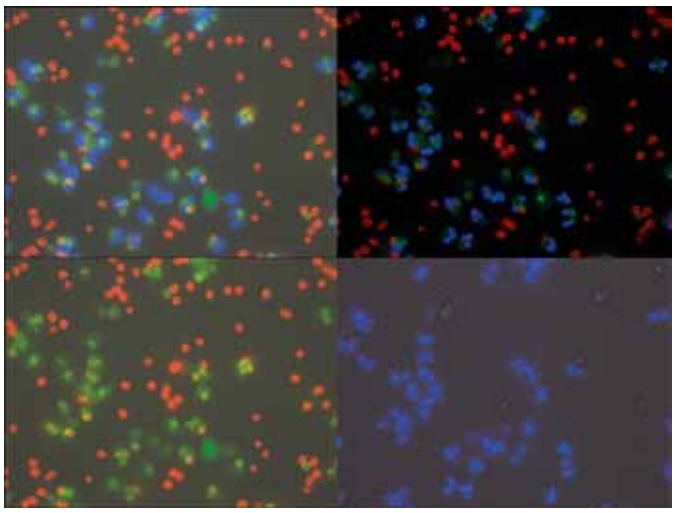

Figure 9
Differential staining of *Candida albicans* phagocytosed by polymorphonuclear granulocytes. Fluorescence labelled *C. albicans* cells appear green inside the neutrophils (nuclei stained with DAPI). Fluorescence of fungal cells outside the phagocyte is quenched with trypan blue (red, false colour image).

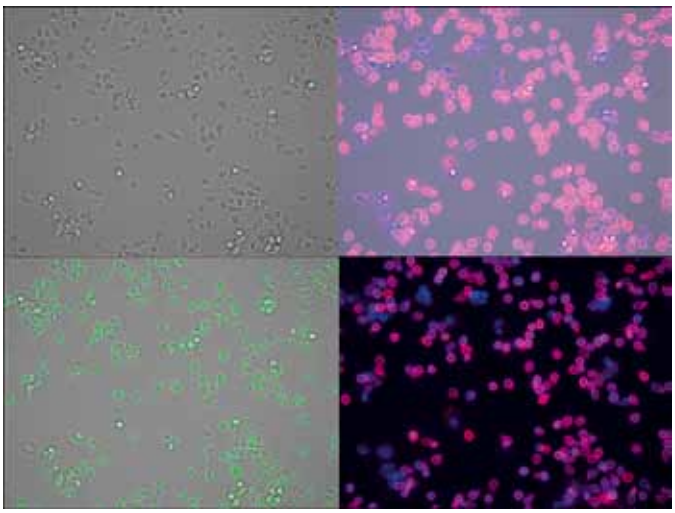

Figure 10
Differential staining of Gfp reporter *C. albicans* strains. Phagocytosed cells are stained with Calcofluor White (CFW, blue) only, while fungal cells outside the phagocyte are stained by both Concanavalin A - Alexa Fluor® 647 (red) and CFW.

References

Ullmann BD et al. (2004) *Eukaryot Cell* 3, 715–723.

Fradin C et al. (2005) *Mol Microbiol* 56, 397–415.

Hromatka BS et al. (2005) *Mol Biol Cell* 16, 4814–4826.

Klippel N et al. (2007) *Analytical Letters* 40, 1400–1411.

Chiranand W et al. (2008) *Eukaryot Cell* 7, 268–278.

the transcriptional response of *C. albicans* in human blood, whereas red cells, mononuclear cells, and plasma have only a minor influence. Gene expression profiles reflect the physiological condition of cells and the micro-environment to which they are exposed. For example, when cells were exposed to red cells, mononuclear cells, or plasma, *C. albicans* genes associated with protein synthesis, glycolysis, and hyphal formation are up-regulated. In contrast, when cells were exposed to neutrophils, genes associated with detoxification of reactive oxygen species, the glyoxylate cycle, and amino acid metabolism are up-regulated. The transcript profile of *C. albicans* in blood reveals two populations of cells, some of which are in contact with neutrophils while the others are not.

How do neutrophils arrest growth and induce oxidative stress? Phagocytosis may lead to the release of antifungal components such as lactoferrin, elastase, defensins, and reactive oxygen species into the phagolysosome. Alternatively, these components could be secreted. Although phagocytosis would seem to explain the starvation response, only 38 % of *C. albicans* cells are phagocytosed. However, exposure to neutrophils strongly up-regulates genes linked to oxidative stress, including genes encoding catalases and superoxide dismutases. These enzymes eliminate endogenous reactive oxygen species accidentally generated during respiration. In pathogenic microbes, they may have an additional function in detoxifying reactive oxygen species caused by the oxidative burst of phagocytic cells.

C. albicans contains as many as six genes (*SOD1-SOD6*) encoding superoxide dismutases, three of which have C-terminal sequences that are typical for proteins attached to the cell membrane or the cell wall. Possibly these enzymes detoxify reactive oxygen species released by phagocytic cells. Our finding that

the surface-associated Sod5 is induced when yeast cells contact or are phagocytosed by neutrophils supports this view.

Transcript profiling shows that neutrophils dominate the host response by confronting *C. albicans* cells with a hostile environment. To survive, the fungus induces genes involved in amino acid biosynthesis pathways, nitrogen metabolism, the glyoxylate cycle, and oxidative stress response. In this case, neutrophils are the key to the host defence against invading *C. albicans* cells. However, the fungus induces a large number of genes to counteract the neutrophils.

The early transcriptional response of human granulocytes to infection with *C. albicans* is not essential for killing but reflects cellular communications. To compare the transcriptional response of leukocytes exposed to *C. albicans*, we investigated the expression of key cytokine genes in polymorphonuclear and mononuclear leukocytes after incubation with *C. albicans* for 1 h. Isolated mononuclear cells expressed high levels of genes encoding proinflammatory signalling molecules, whereas neutrophils exhibited much lower levels, similar to those observed in whole blood. The global transcriptional profile of neutrophils was examined by using an immunology-biased human microarray to determine whether different morphological forms or the viability of *C. albicans* altered the transcriptome. Hyphal cells appeared to have the broadest effect, although the most strongly induced genes were regulated independently of morphology or viability. These genes were involved in proinflammatory cell-cell signalling, cell signal transduction, and cell growth. Generally, genes encoding known components of neutrophil granules showed no up-regulation at this time point; however, lactoferrin, a well known candidacidal peptide, was secreted by neutrophils. Addition of inhibitors of RNA or protein de novo synthesis did not influence the killing activity within 30 min. These results support the general notion that neutrophils do not require gene transcription to mount an immediate and direct attack against microbes. However, neutrophils exposed to *C. albicans* express genes involved in communication with other immune cells (Fradin *et al.*, 2005).

Investigating interactions of *C. albicans* with neutrophils using mutants and GFP-reporter strains

Neutrophils play a major role as a first line of defence against any microbial pathogen. Despite that blood is a hostile environment, *C. albicans* can disseminate in the host via the bloodstream and can cause life threatening systemic infections and sepsis in immunocompromised individuals. Previous results have shown that neutrophils govern the transcriptional response, morphology and proliferation of *C. albicans* in human blood. Neutrophils evoke a carbohydrate- and nitrogen-starvation and induce genes involved in reactive oxygen- and nitrogen-species detoxification.

We aim to elucidate how *C. albicans* is recognised, attacked and killed by neutrophils and how the fungus responds to overcome the attack by neutrophils. To identify genes and factors necessary to counteract neutrophils, several selected mutants have been tested in their response towards neutrophils by means of a set of damage assays (XTT; propidium iodide-based) and CFU counting.

By screening our mutant collection with the XTT assay, we identified several mutants with modified sensitivity towards neutrophils. For example, mutants *als3*Δ, *hgc1*Δ, *pra1*Δ exhibited increased sensitivity when grown as yeast cells, and *rim101*Δ both during yeast and hyphal growth. In contrast, *bud2*Δ, *ecm33*Δ, *eed1*Δ, *pmt2*Δ/*PMT2* were more sensitive towards neutrophils both as yeast and hyphae, while *pra1*Δ and *tec1*Δ only as hyphae.

Production of reactive nitrogen species is one of several ways neutrophils attack pathogens. From our transcriptional profiling of *C. albicans* during interaction with blood components, we monitored a transcriptional response to overcome nitrosative stress. *YHB1* encodes a scavenging flavohaemoglobin that transforms toxic nitric oxide into harmless nitrate (Ullmann *et al.*, 2004; Hromatka *et*

al., 2005), whereas *CTA4* codes for the transcription factor that controls the expression of YHB1 (Chiranand *et al.* 2008). Therefore, we investigated whether mutants lacking these components of the nitrosative stress response (*cta4*Δ and *yhb1*Δ) exhibit increased sensitivity towards neutrophils. Using our propidium iodide-based viability protocol, we observed that these mutants have modified sensitivity towards neutrophils. To further confirm these findings, these mutants were tested in a killing assay and survival was tested by counting CFUs. Surprisingly, *cta4*Δ was not more sensitive than the wild type strain SC5314, while *yhb1*Δ exhibited increased sensitivity.

Phagocytosis is extremely important for the clearance of pathogens by phagocytes. Therefore, we have established a set of differential staining protocols to monitor phagocytosis.

Furthermore, we use Gfp reporter strains to monitor the transcriptional response of *C. albicans* in response to either extracellular attack or phagocytosis on a single cell level, indicating the expression of marker genes known to be up-regulated during carbohydrate starvation (*ICL1*, *MLS1*), nitrogen starvation (*GCN4*, *MEP2*), oxidative stress (*CTA1*, *SOD5*, *TTR1*, *TRX1*), nitrosative stress (*CTA4*, *YHB1*), or pH changes (*PRA1*, *PHR1*, *PHR2*). Using *MEP2*- and *PRA1*-driven Gfp reporter strains, we could show that expression of these genes during interaction with neutrophils is contact dependent, confirming the up regulation of ammonium uptake systems during interaction with neutrophils, as well as changes in the pH sensed by *C. albicans*. Further work will focus on discerning whether phagocytosis is needed to induce Gfp expression or contact is sufficient in these and relevant reporter strains.

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9 *In vivo* infection models

Group Leaders: Ilse Jacobsen, Katharina Große, Silvia Slesiona

The use of suitable models for the analysis of virulence and pathogenicity is an important part in the investigation of pathogenic fungi and other microbial pathogens.

Murine models are the gold standard for testing fungal pathogenesis to date and are commonly used to study the role of individual fungal genes during infection. Therefore, mice are infected with defined fungal knockout mutants. However, before fungal mutants are tested *in vivo*, selected *in vitro* and *ex vivo* models should be used to gain some insight into the phenotypic characteristics of these mutants. This allows us to choose the right model to test attenuation of mutants.

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

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

To enable in-depth analysis of *in vivo* experiments, we established methods to monitor blood parameters, analyse cytokine responses and routinely perform histology to evaluate fungal morphology, quality of tissue damage and the immune response of the host. Processing of tissue samples for histopathology is done in collaboration with the Fritz-Lippmann-Institute (FLI) at the Beutenberg campus.

Invasive Aspergillosis in mice

Invasive aspergillosis is a life-threatening disease in immunocompromised patients. In order to elucidate pathogenicity mechanisms of both the host and the pathogen, murine models have been developed. Mice are only susceptible to *Aspergillus* infections if immunocompromised. We use both immunosuppresion by cyclophosphamide (panleukopenic) and by cortisone (reduced phagocyte function) to mimic different kinds of immunosuppression that put human patients at risk for invasive apergillosis. The animals are infected intranasally under general anaesthesia.

Infections with *Candida albicans*

C. albicans infections can manifest at different body sites and range from inconvenient but comparably harmless local infections to severe and life threatening disseminated candidiasis. To adress these different manifestations, different murine models have been developed.

To study invasion properties of *C. albicans* mutants, which were demonstrated to be reduced in invasion in vitro, we use intraperitoneal infections. This model causes little distress for the animals and is therefore a valuable substitute for mortality studies.

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

Development of systemic a *Candida glabrata* infection model in mice

Disseminated infections with *C. glabrata* are less common than infections with *C. albicans* but due to the higher resistance of *C. glabrata* to azoles more difficult to treat. Furthermore, the pathogenesis of *C. glabrata* infections is only poorly understood. Mammalian infection models are crucial for understanding pathogenesis of *C. glabrata*. However, no standardised infection model for *C. glabrata* has been described yet. The murine *C. glabrata* models described in the literature vary greatly in respect to mice strains, infectious dose and immunosuppression. The common feature of all literature reports is that *C. glabrata* does not cause clinical disease in wild type mice. Analysis of infection experiments therefore relies on quantitative reisolation and histology.

We recently established a protocol for systemic murine *C. glabrata* – infection as part of a work package of the ERA-NET PathoGenoMics Program: "Genomic Approaches to unravel the molecular basis of pathogenicity in the Human Fungal Pathogen *Candida glabrat*a – FunPathCaGla". In this study (Jacobsen *et al*., 2009) we showed that leucopenia, but not treatment with corticosteroids, leads to transiently increased fungal burdens in comparison to immunocompetent mice. However, even immunocompetent mice were not capable of clearing infections within four weeks. Tissue damage and immune responses to microabscesses were mild as monitored by clinical parameters, including blood enzyme levels, histology, myeloperoxidase and cytokines levels. Furthermore, we investigated the suitability of amino acid auxotrophic *C. glabrata* strains for *in vitro* and *in vivo* studies on fitness and or virulence. Histidine, leucine or tryptophan auxotrophy, as well as a combination of these auxotrophies, did not influence *in vitro* growth in rich medium. Survival of all auxotrophic strains in immunocompetent mice was similar to the parental wild type strain during the first week of infection and only mildly reduced four weeks after infection, suggesting that *C. glabrata* is capable of utilizing a broad range of host-derived nutrients during infection. These data suggest that *C. glabrata*

References

Fradin F and Hube B (2006) *Microbe* 2, 1–5.

Fradin C et al. (2007) *Infect Immun* 75, 1493–1501.

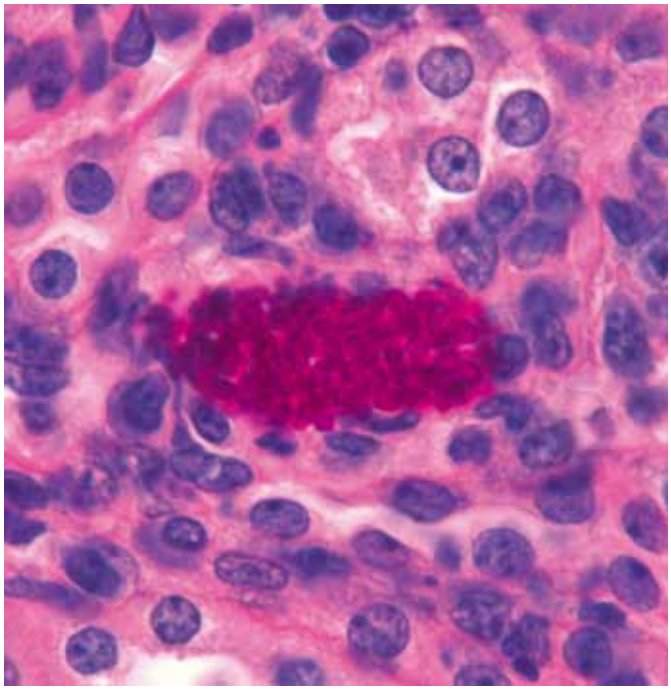

Figure 11
Histological kidney section from a mouse infected with *Candida glabrata*. Fungal cells are visible in pink as a microcolony within the tubuli.

Figure 12
PET/CT image of a mouse experimentally infected with *Aspergillus fumigatus*. Immune cell infiltration is visualized by enrichment of the radioactive tracer in the right lung (white arrow).

References
Jacobsen ID et al. (2009) *Infect Immun*, published ahead of print on 14 December 2009, doi:10.1128/IAI.01244–09.

histidine, leucine or tryptophan auxotrophic strains are suitable for generation of knockout mutants for *in vivo* studies. Notably, our work indicates that *C. glabrata* has successfully developed immune evasion strategies to survive, disseminate and persist within mammalian hosts.

Development of murine infection models for *Aspergillus terreus*

In contrast to *A. fumigatus*, no well characterized murine infection models for *A. terreus* have been described. As part of a collaboration project with the Junior research group Microbial Biochemistry and Physiology (Matthias Brock) at the HKI we established a leucopenic and a cortisone based pulmonary infection model for *A. terreus*. Initial characterization indicates interesting differences in pathogenesis of *A. fumigatus* and *A. terreus*. We currently perform in-depth characterization of infections caused by these two related fungi.

In vivo imaging as a tool to reduce and refine animal models

In vivo imaging can be used to directly monitor the progression of infections and inflammation in animals over time. By using such methods it is no longer necessary to sacrifice animals at given time points, thus reducing the number of animals needed to analyse the infection progress over time. We are currently establishing positron emission tomography/computer tomography (PET/CT) to study the progression and magnitude of inflammation and fungal burden during invasive aspergillosis and disseminated candidiasis in mice.

In both animal models, the host's inflammatory response, especially immune cell infiltrations in target organs, contributes significantly to pathogenesis. Therefore, we analysed the suitability of a radioactive tracer which accumulates in metabolically active cells, e. g. immune cells, to visualize the immune response during infection *in vivo*. We were able to detect

PET signals in the affected organs of infected mice and are currently determining whether this accumulation can be used quantitatively to measure the immune response. Therefore, the PET/CT data is compared with molecular markers of infection such as myeloperoxidase and various cytokines.

The second approach is to visualize the fungal burden *in vivo* using PET/CT. For this purpose, radioactively labelled compounds which bind to the surface of fungal cells are currently developed by Peter Gebhard from the Department of Cell and Molecular Biology (Hans-Peter Saluz).

Alternative infection models

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

Embryonated eggs are used as an alternative model providing a highly complex environment, including phagocytic cells, to study fungal host-pathogen interaction, but are of a lower developmental stage than adult mice. We could show that embryonated eggs are suitable to test the virulence of *A. fumigatus* and *C. albicans* mutants with good correlation to the gold standard mouse models. The model can be analysed using re-isolation, macroscopic and microscopic analysis of pathological alterations and the analysis of the hosts immune response by determining the transcription levels of cytokines and chemokines.

Using these techniques, we found strong indications that the main cause of death in embryos infected by *A. fumigatus* is invasion and destruction of host tissue at the site of infection. The susceptibility to infection depends on the age of the embryo at the time of infection – older embryos are more resistant. This is likely due to the maturation of the immune system. As embryos are highly susceptible to infection, immunosuppressive regimens are not required in this model; however, by choosing embryos at a different age, the different immunosuppressed mouse models can be mimicked.

In systemic murine *C. albicans* infection models the host's immune response contributes significantly to the outcome of infection. Virulent *C. albicans* strains induce a septic shock which leads to death. We could recently show that similar mechanisms determine the outcome in infected embryos. The induction of cytokine storm in embryos is linked to the strain's ability to invade the host tissue. Thus, the embryonated egg model can be used to investigate the ability of *C. albicans* mutants to invade and induce excessive cytokine production *in vivo*.

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Deutsche Forschungsgemeinschaft
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Bernhard Hube, Matthias Brock

Deutsche Forschungsgemeinschaft
Schwerpunktprogramm 1160: Kolonisation und Infektion durch humanpathogene Pilze
Teilprojekt: Identification and characterisation of virulence associated genes during oral infections with *Candida albicans*
Bernhard Hube

Deutsche Forschungsgemeinschaft
Cell surface associated secreted aspartic proteases (saps) of *Candida albicans*
Bernhard Hube

Bundesministerium für Bildung und Forschung
ERA-NET PathoGenoMics
FunPath: Genomweite Ansätze zur Aufklärung der molekularen Pathogenitätsmechanismen des Humanpathogens *Candida glabrata*
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Bundesministerium für Bildung und Forschung
PET/CT zur Darstellung von Infektion und Entzündung: Reduction, Refinement und Replacement von Tierversuchen
Ilse Jacobsen, Hans Peter Saluz

European Union
6. Forschungsrahmenprogramm der EU
Marie Curie Research Training Networks
CaInfectome: Unravelling the infectome of *Candida albicans*
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European Union
6. Forschungsrahmenprogramm der EU
Marie Curie Research Training Networks
FINSysB: Pathogenomics and systems biology of fungal infections - an integrative approach
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Selected publications
(HKI authors in bold)

Almeida RS, **Brunke S**, **Albrecht A**, Thewes S, Laue M, Edwards JE, Filler SG Jr, **Hube B** (2008) The hyphal-associated adhesin and invasin Als3 of *Candida albicans* mediates iron acquisition from host ferritin. *PLoS Pathogens* 4, e1000217.

Wozniok I, Hornbach A, Schmitt C, Frosch M, Einsele H, **Hube B**, Löffler J, Kurzai O (2008) Induction of ERK kinase signalling triggers morphotype specific killing of *Candida albicans* filaments by human neutrophils. *Cellular Microbiology* 10, 807-820.

Naglik JR, Moyes D, Makwana J, Kanzaria P, Tsichlaki E, Weindl G, Tappuni AR, Rodgers CA, Woodman AJ, Challacombe SJ, Schaller M, **Hube B** (2008) Quantitative expression of the *Candida albicans* secreted aspartyl proteinase gene family in human oral and vaginal candidiasis. *Microbiology* 154, 3266-3280.

Wilson D, Thewes S, Zakikhany K, Fradin C, **Albrecht A**, **Almeida R**, **Brunke S**, **Grosse K**, **Martin R**, **Mayer F**, **Leonhardt I**, **Schild L**, **Seider K**, **Skibbe M**, **Slesiona S**, **Wächtler B**, **Jacobsen I**, **Hube B** (2009) Identifying infection-associated genes of *Candida albicans* in the post genomic era. *FEMS Yeast Research* 9, 688-700.

Meiller TF, **Hube B**, **Schild L**, Shirtliff ME, Scheper MA, Winkler R, Ton A, Jabra-Rizk MA (2009) A novel immune evasion strategy of *Candida albicans*: proteolytic cleavage of a salivary antimicrobial peptide. *PLoS ONE* 4, e5039.

Butler G, Rasmussen MD, Lin MF, Santos MAS, Sakthikumar S, Munro CA, Rheinbay E, Grabherr M, Forche A, Reedy JL, Agrafioti I, Arnaud MB, Bates S, Brown AJP, **Brunke S**, Costanzo MC, Fitzpatrick DA, de Groot PWJ, Harris D, Hoyer LL, **Hube B**, Klis FM, Kodira C, Lennard N, Logue ME, **Martin R**, Neiman AM, Nikolaou E, Quail MA, Quinn J, Santos MC, Schmitzberger FF, Sherlock G, Shah P, Silverstein K, Skrzypek MS, Soll D, Staggs R, Stansfield I, Stumpf MPH, Sudbery PE, Thyagarajan S, Zeng Q, Berman J, Berriman M, Heitman J, Gow NAR, Lorenz MC, Birren BW, Kellis M, Cuomo CA (2009). Evolution of pathogenicity and sexual reproduction in eight *Candida* genomes. *Nature* 459, 657-662.

Dalle F, **Wächtler B**, Coralie L'O, Holland G, Bannert N, **Wilson D**, Labruère C, Bonnin A, **Hube B** (2009) Cellular interactions of *Candida albicans* with human oral epithelial cells and enterocytes. *Cellular Microbiology* 12, 248-271.

Hube B (2009) Fungal adaptation to the host environment. *Current Opinion in Microbiology* 12, 1-3.

Wilson D, **Hube B** (2010) Hgc1 mediates dynamic *Candida albicans*-endothelium adhesion events during circulation. *Eukaryotic Cell* 9, 278-287.

Jacobsen ID, **Brunke S**, **Seider K**, Schwarzmüller T, Firon A, d'Enfert C, Kuchler K, **Hube B** (2010) *Candida glabrata* persistence in mice does not depend on host immunosuppression and is unaffected by fungal amino acid auxotrophy. *Infect Immun*, 78, 1066-1077.

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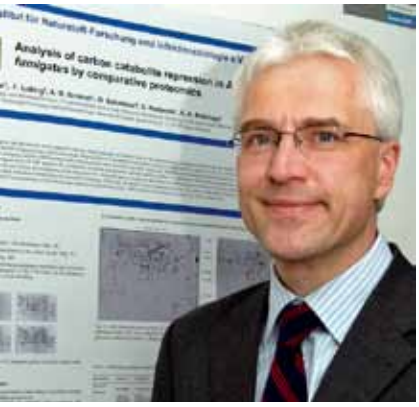

The Department of Molecular and Applied Microbiology is devoted to research in the two main areas of the Leibniz Institute of Natural Product Research and Infection Biology (HKI), i.e., infection biology of fungi and natural product research. Furthermore, for the first time genomes of dermatophytic fungi were sequenced and characterised.

1. Pathobiology of *Aspergillus fumigatus*

During the past 20 years, the incidence of fungal infections in humans has risen considerably. This increase in infections is associated with excessive morbidity and mortality and is directly related to increasing patient populations at risk for the development of serious fungal infections, which includes individuals undergoing solid-organ transplantation, blood and marrow transplantation, major surgery, and those with AIDS, neoplastic disease, immunosuppressive therapy, advanced age, and premature birth. Fungal infections are categorised in two groups: topical and systemic infections. Topical fungal infections affect body surfaces and can be chronic. Systemic fungal infections can occur in an organ or in the whole body and are transferred via the bloodstream. Compared to other microbial infections, systemic fungal infections are characterised by lower frequencies but generally high mortality rates (30–90%).

Aspergillus fumigatus has become the most important airborne fungal pathogen of hu-

INTRODUCTION | EINLEITUNG

Head:
Prof. Dr. Axel A. Brakhage

Die Abteilung Molekulare und Angewandte Mikrobiologie befasst sich mit den beiden Forschungsschwerpunkten des HKI, der Naturstoff-Forschung und Infektionsbiologie. Die Abteilung konzentriert sich dabei auf die beiden Pilzspezies *Aspergillus nidulans* und *A. fumigatus*. Außerdem wurden erstmalig Genome von Dermatophyten sequenziert und charakterisiert.

1. Pathobiologie von *Aspergillus fumigatus*

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

mans. Diseases caused by *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 %.
Research at the Department covers all relevant aspects of the pathobiology *A. fumigatus*. Research includes the areas of signal transduction, elucidation of unusual pathways like the biosynthesis of melanins, improvement of genetic tools, genomics, proteomics, transcriptomics, pathogen/host (immune effector cells) interaction with emphasis on alveolar macrophages and neutrophilic granulocytes. Furthermore an animal model is available. Based on results obtained in the Department, the identified proteins involved in virulence will be evaluated as target proteins for antifungal drugs or the development of immune therapies.

2. Microbial communication and activation of silent fungal secondary metabolism gene clusters

Fungi produce numerous of secondary metabolites. Some of these compounds are used as antibiotics such as the β-lactams penicillin

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 schwierig, die Möglichkeiten einer Therapie sind begrenzt, so dass die invasive Aspergillose nach wie vor durch eine sehr hohe Letalitätsrate gekennzeichnet ist.
Die Mitarbeiter der Abteilung beschäftigen sich mit allen Aspekten der Pathobiologie von *A. fumigatus*. Die Forschungsarbeiten beinhalten die Gebiete Signaltransduktion, spezielle Stoffwechselwege wie Melaninbiosynthesen, die Verbesserung gentechnischer Analysemethoden, Genomik, Proteomik, Transkriptomik sowie das Studium von Pathogen/Wirt-Wechselbeziehungen, wie der Interaktion von *A. fumigatus* mit alveolaren Makrophagen und neutrophilen Granulozyten. Für Infektionsstudien ist ein Tiermodell verfügbar. Identifizierten Proteine werden als potentielle Targets für neue antifungale Wirkstoffe oder die Entwicklung von Immuntherapien intensiv untersucht.

2. Mikrobielle Kommunikation und Aktivierung stiller pilzlicher Sekundärmetabolismus-Gencluster

Pilze produzieren eine Vielzahl sekundärer Stoffwechselprodukte, von denen viele als Antibiotika genutzt werden, wie zum Beispiel die β–Lactame Penicillin und Cephalosporin oder Immunsuppressiva wie Cyclosporin. Andere Metabolite scheinen für die Virulenz human-

and cephalosporin, or as immunosuppressants like cyclosporin. Others have been proposed to be important for virulence e.g. of the human-pathogenic fungus *A. fumigatus*.

In collaboration with the Department of Biomolecular Chemistry, the Bio Pilot Plant and the research group Systems Biology/Bioinformatics, genome mining technologies have been applied to identify and discover silent fungal secondary metabolism gene clusters. This led to the discovery of novel compounds. Recently, we elucidated a novel principle of activation based on the interaction of a fungus and a bacterium. Cocultivation of *A. nidulans* with *Streptomyces hygroscopicus* led to the activation of a silent gene cluster and the production of a novel compound by the fungus.

Research has been aimed at the elucidation of regulatory circuits, extra- and intracellular signals and mechanisms leading to the activation of distinct areas on the chromosomes encoding secondary metabolism gene clusters. These technologies will have a major impact on future drug discovery programs.

3. Eukaryotic transcription factors

Some projects of the Department focus on the elucidation of molecular mechanisms regulating transcription factors with emphasis on transcription factors involved in fungal secondary metabolism. This research led to the discovery of a novel mechanism of iron and redox regulation in eukaryotes based on the CCAAT binding complex and a novel subunit

pathogener Pilze bedeutsam zu sein. Mit Hilfe von neuartigen „Genome mining"-Strategien werden in Kooperation mit der Abteilung Biomolekulare Chemie, dem Biotechnikum und der Forschungsgruppe Systembiologie/Bioinformatik stille Gencluster von *A. nidulans* aktiviert und neue Wirkstoffe charakterisiert. Besonders wichtig in diesem Zusammenhang ist das Ergebnis, dass die Interaktion von Pilzen mit Bakterien zur Aktivierung stiller Gencluster und zur Bildung neuer Substanzen durch den Pilz führt. Der Signalaustausch, die Signaltransduktion sowie die Mechanismen der Aktivierung bestimmter chromosomaler Abschnitte stehen im Vordergrund der laufenden Untersuchungen, sowie die Isolierung neuer Substanzen durch die Kombination von *genome mining* und Genaktivierungsstrategien. Insgesamt handelt es sich dabei um ein spannendes, sich schnell entwickelndes Wissenschaftsfeld zur Identifizierung neuer Wirkstoffe.

3. Eukaryotische Trankriptionsfaktoren

Einige Projekte der Abteilung befassen sich mit der Aufklärung neuer molekularer Mechanismen der Regulation insbesondere von pilzlichen Sekundärmetabolismus-Genclustern. So konnte der CCAAT-Bindekomplex weiter charakterisiert und neue Mechanismen der Eisen- und Redoxregulation konnten in Eukaryoten entdeckt werden. Dazu werden Oberflächen-Plasmon-Resonanz-Analysen zur Untersuchung von Protein/Protein- und Protein/DNA-Wechselwirkungen, und umfangreiche proteinchemische Methoden inklusive Proteomanalysen eingesetzt.

designated as HapX. Surface plasmon resonance analyses have been applied for the investigation of protein/protein and protein/DNA interactions as well as comprehensive protein chemical techniques including proteome analyses.

1 Proteomics of filamentous fungi

Group Leaders: Olaf Kniemeyer,
Axel A. Brakhage

Analysis of the fungal proteome of *Aspergillus fumigatus*, *Aspergillus nidulans* and *Arthroderma benhamiae*

In the Department of Molecular and Applied Microbiology we have focused our research on *Aspergillus nidulans* and *Aspergillus fumigatus*. Species of *Aspergillus* are medically and commercially very important. For example, *A. fumigatus* has become the most important airborne fungal pathogen and *A. nidulans* has been used as model organism in microbiology and cell biology for many years. After sequencing of the genomes of both fungal species it is possible to study protein regulation on a global scale. One of the most suitable protein separation techniques is 2D-gel electrophoresis, which is based on separation of proteins by charge and size in a gel matrix. This method enables the separation and visualisation of a substantial fraction of an organism's complete set of proteins, the proteome. We use this method to gain a comprehensive overview about the proteins present or induced during environmental changes and stress conditions which are relevant for important biological regulation circuits and pathogenicity. For example, the acquisition of iron is known to be an essential step in microbial infections due to iron-limiting conditions in the human host. To understand the cellular processes induced by iron starvation, we analysed the proteome of *A. fumigatus* grown under iron-deficiency conditions. Many enzymes containing iron as cofactor were down-regulated, whereas among the up-regulated proteins two so far uncharacterised proteins were found. Molecular biology experiments are under way to elucidate their roles during iron depletion.

In 2007, the sequencing of the zoophilic dermatophyte *Arthroderma benhamiae* has been initiated at the HKI in close collaboration with the Leibniz Institute for Age Research, Jena, and the Friedrich Schiller University Jena and was completed in 2009. It is estimated that 30 % of all humans are infected by dermatophytes, a group of fungi which infect keratinised tissue such as skin and nails. Knowledge about the biology and pathobiology of this group of fungi is very limited. Therefore, we have extended the scope of our research on the proteome of this important dermatophyte. In the following paragraphs we report some exemplary research projects of the Research group "Fungal Proteomics".

Two-dimensional proteome reference maps for the human pathogenic filamentous fungus *Aspergillus fumigatus*.

In recent years, *A. fumigatus* has attracted more and more attention as the major cause of life-threatening lung infections in immunocompromised patients, known as invasive aspergillosis (IA). IA is still associated with mortality rates up to 60–90 %. The mechanisms leading to pathogenicity of *A. fumigatus* are poorly understood. No single clear and obvious virulence factors could be detected yet, but it is generally accepted that *A. fumigatus* obviously possesses higher stress tolerance and better mechanisms to adapt to the host environment in comparison to other fungi. We established a 2-D reference map for *A. fumigatus*. Such type of proteome maps provide information about all the proteins, which are synthesized under standard cultivation conditions and which are accessible to 2D-gel electrophoresis. After 2D-gel electrophoresis, all visible protein spots were excised, cleaved in smaller peptides by the protease trypsin and were further analyzed by mass spectrometry using MALDI-TOF-MS/MS. Altogether, we identified 381 spots representing 334 different proteins. Proteins involved in cellular metabolism, protein syn-

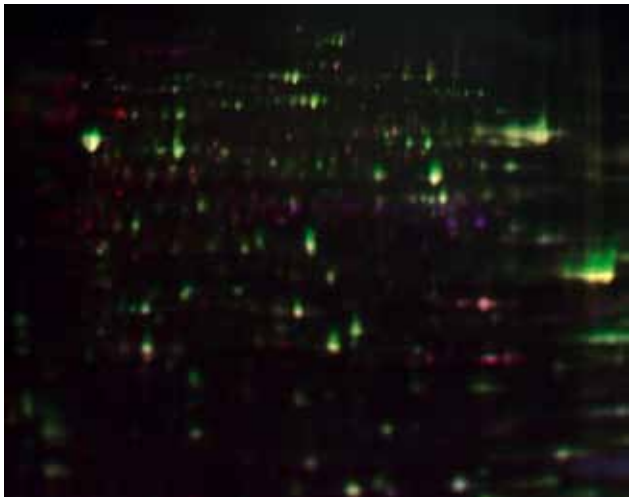

Figure 1
Proteome map of *A. fumigatus* mitochondrial proteins.

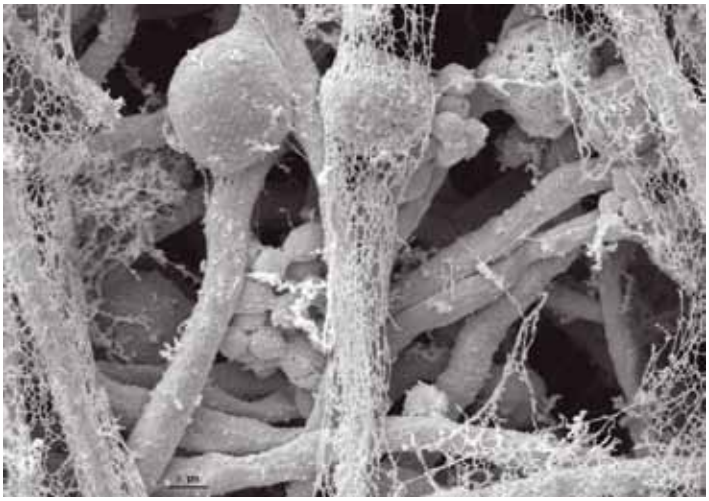

Figure 2
Scanning electron microscopy (SEM) micrograph of *A. fumigatus* hyphae trapped in neutrophil extracellular traps (NETs) (Bruns et al. 2010).

thesis, transport processes and cell cycle were most abundant. Furthermore, we established a protocol for the isolation of mitochondria of *A. fumigatus* and developed a mitochondrial proteome reference map as well. Mitochondria are the "cellular power plants" which generate the cell's chemical energy. In addition, they are also involved in many other important cellular functions, such as signaling, cell growth and cell death. 147 proteins represented by 234 spots were identified. These proteome maps constitute powerful tools for further biological studies of *A. fumigatus* stress response, pathogenicity and the characterization of *A. fumigatus* antigens.

Surface hydrophobin prevents immune recognition of airborne fungal spores.

The air we breathe is filled with thousands of fungal spores, the reproductive structure of fungi. The spore surface is often covered by a thin layer of regularly arranged rodlets. These rodlets are composed of specific proteins, which are designated rodlet proteins. The rodlet layer confers water repellency and probably protects the spores against dehydration. In *A. fumigatus*, this rodlet layer is composed of the RodA protein, which is covalently bound to the conidial cell wall. In collaboration with the Institute Pasteur (Jean-Paul Latgé), the University of Perugia (Luigia Romani) and University Paris Descartes we could assign an additional function to the rodlet layer. It masks the recognition of the spore surface structures by the immune system and hence prevents an immune response. When the RodA protein was extracted from the spore surface by chemical cleavage using fluoric acid and incubated with phagocytic cells of the immune system (alveolar macrophages and dendritic cells) it did not trigger an immune response. In contrast, mutants with a deletion in the *rodA* gene induced an immune activation. Taken together, these observations showed that the rodlet layer of the spore surface immunologically silences airborne moulds and thus do not activate the

host innate immune cells continuously and do not induce detrimental inflammatory responses upon inhalation. The avoidance of immune activation has benefits for *A. fumigatus* as well as the host. For the host it prevents chronic inflammation in the lungs, whereas for the fungus it impedes rapid elimination by the host's immune system.

Neutrophil extracellular traps

Inhaled *A. fumigatus* spores (conidia) are in general cleared in the human host by phagocytic cells of the innate immune system, which provides an immediate, but non-specific response. The phagocytes, such as macrophages or neutrophils, recognize, engulf and subsequently kill fungal conidia. Hyphae, the vegetative growth form of a fungus, are too large to be engulfed. Neutrophils play presumably the major role in attacking the large and long hyphae of filamentous fungi through close contact and secretion of anti-microbials. However, the extracellular killing mechanisms of *A. fumigatus* hyphae by neutrophils have not been clarified yet. The recently discovered neutrophil extracellular traps (NETs) may be another important factor for the clearance of pathogenic filamentous fungi. NETs consist of nuclear DNA, which forms an extracellular fibril matrix decorated with antimicrobial proteins. Upon stimulation by a pathogen, a cell death program is activated: the nuclei of neutrophils loose their shape, first the nuclear envelope and finally the cell membrane disintegrate and DNA is released into the extracellular space. When we started our project on NET formation in response to *A. fumigatus* the importance of neutrophils for their production *in vivo* was unclear and the molecular mechanisms of the fungus to avoid or at least reduce NET-formation was hardly known. We showed in confrontation assays that human neutrophils produce NETs *in vitro* when encountering *A. fumigatus*. In close collaboration with the University of Magdeburg (Matthias Gunzer) we produced time-lapse movies which showed that NET production was a highly dynamic process which, however, was only exhibited by a sub-population of cells. NETosis was maximal against hyphae, but reduced against resting and swollen conidia. In a newly developed mouse model we demonstrated the existence and measured the kinetics of NET-formation *in vivo* by 2-photon microscopy of *Aspergillus*-infected lungs. The two-photon excitation microscopy is a fluorescence imaging technique that allows imaging living tissue. Neutrophil depletion in mice by specific antibodies almost completely inhibited NET-formation in lungs, thus providing direct evidence that indeed neutrophils form NETs *in vivo* during infection with *A. fumigatus*. We also demonstrated that the above mentioned conidial rodlet layer consisting of the surface protein RodA inhibits NET formation when neutrophils encounter *Aspergillus* fungal elements. Altogether, our data suggest that NETs may have a fungistatic effect, but do not directly affect killing of fungal hyphae at least in the first 8 hours.

2 Virulence of *Aspergillus fumigatus*

Group Leaders: Thorsten Heinekamp, Axel A. Brakhage

Characterisation of G protein-coupled receptors of *Aspergillus fumigatus*

The filamentous fungus *Aspergillus fumigatus* normally grows on compost or hay but is also able to colonise environments such as the human lung. In order to survive, this organism needs to react to a multitude of external stimuli. Although extensive work has been carried out to investigate intracellular signal transduction in *A. fumigatus*, little is known about the specific stimuli and the corresponding receptors activating these signaling cascades. We characterised two putative G protein-coupled receptors, GprC and GprD, with respect to their cellular functions. Deletion of the corresponding genes resulted in drastic growth defects as hyphal extension was reduced, germination was retarded and hyphae showed elevated levels of branching. The growth defect was found to be temperature dependent. The higher the temperature the more pronounced was the growth defect. Furthermore, compared with the wild type the sensitivity of the mutant strains towards environmental stress caused by reactive oxygen intermediates was increased and they dis-

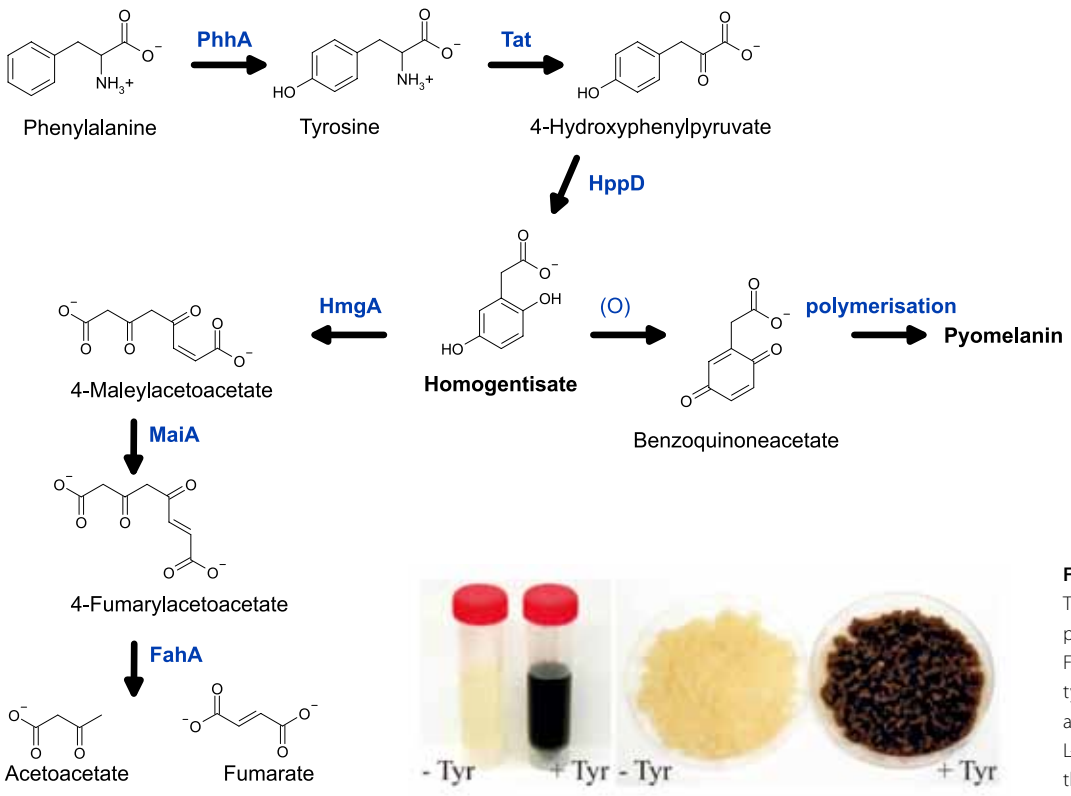

Figure 3
Tyrosine degradation pathway and pigment formation in *A. fumigatus*. For pyomelanin formation the wild type was grown for 3 days with (+Tyr) and without (-Tyr) addition of 10 mM L-tyrosine. Pigment was observed in the mycelium and in the medium.

played an attenuation of virulence in a murine infection model. Transcriptome analyses indicated that in the deletion mutants of both *gprC* and *gprD*, transcripts of primary metabolism genes were less abundant, whereas transcription of several secondary metabolism gene clusters was upregulated. Our data suggest the receptors are involved in integrating and processing stress signals.

Cell wall integrity signalling and pyomelanin formation

Mitogen-activated protein kinase (MAPK) signalling pathways are involved in the regulation of various cellular responses to environmental changes in eukaryotes. Genome Blast analysis revealed that the central core of the cell wall integrity signalling pathway in *A. fumigatus* is composed of three protein kinases designated Bck1, Mkk2 and MpkA. This pathway is of particular interest because it represents a possible target for new antifungal drugs. Deletion of these genes resulted in severe sensitivity of the mutants against cell wall disturbing compounds and drastic alterations of the fungal morphology. Western blot analysis demonstrated that Bck1 and Mkk2 directly activate MpkA during vegetative growth and under cell wall stress conditions further confirming that Bck1, Mkk2 and MpkA form a MAP kinase module. Interestingly, this MAP kinase module affects the formation of pyomelanin derived from tyrosine degradation.

Melanins are implicated in the pathobiology of a broad range of human and plant pathogenic fungi. Apart from dihydroxynaphthalene (DHN)-melanin, *A. fumigatus* is able to synthesise a brown pigment, when L-tyrosine is present in the media. A proteomic approach implied that the formation of this L-tyrosine derived brown compound is related to proteins involved in the tyrosine/phenylalanine degradation pathway, in which homogentisate is the major intermediate. Homogentisic acid

is the primary precursor of melanin in a broad range of microorganisms. Therefore, we analysed the homogentisate degradation pathway in *A. fumigatus*. The genes, coding for enzymes involved in the homogentisate degradation pathway form a cluster. Deletion of the genes coding for central enzymes of the pathway, i.e., homogentisate dioxygenase (*hmgA*) and 4-hydroxyphenylpyruvate dioxygenase (*hppD*), led to the identification of pyomelanin that is formed as brown pigment by polymerization of homogentisic acid. In a Δ*hmgA* strain, homogentisic acid accumulates and pyomelanin production is increased in contrast to the Δ*hppD* mutant strain that does not produce homogentisic acid. We showed that HppD and HmgA are involved in scavenging of reactive oxygen species (ROS), which implies that accumulation of homogentisate in *A. fumigatus* plays an important role not only for production of pyomelanin, but also for the virulence of this fungal pathogen.

Interaction of *Aspergillus fumigatus* with the immune system

Innate immunity plays an important role in the defense against infections. The complement system represents an essential part of the innate immune system. This cascade system is activated on the surface of *A. fumigatus* conidia and hyphae and enhances phagocytosis of conidia. *A. fumigatus* conidia but not hyphae bind host complement regulators factor H, FHL-1 and CFHR-1 to their surface which control complement activation. In close collaboration with the group of P. Zipfel (Department of Infection Biology, HKI) we showed that *A. fumigatus* hyphae possess an additional endogenous activity to control complement activation. *A. fumigatus* culture supernatant efficiently cleaved complement components C3, C4, C5, C1q as well as immunoglobulin G. Secretome analysis and protease inhibitor studies identified the secreted alkaline protease Alp1, which is present in high amounts in the culture supernatant, as the central molecule responsible for this cleavage. An *alp1* deletion strain was generated and the culture supernatant possessed minimal complement degrading activity. Thus, Alp1 is responsible for the observed cleavage and degrades a broad range of different substrates. This novel mechanism further enables *A. fumigatus* to evade from the host complement attack.

3 Transcription factors and signal transduction / Protein-protein interactions

Group Leaders: Peter Hortschansky, Axel A. Brakhage

Regulation of fungal transcription factor complexes

The filamentous fungus *Aspergillus nidulans* is an excellent model to study many fundamental biological questions which includes development, hyphal growth or the production of secondary metabolites. The fungus propagates sexually via the formation of cleistothecia and ascospores or asexually by production of conidia (spores). In addition, the fungus is characterised by vegetative, hyphal growth. Since we have been interested in redox regulation and cellular differentiation, signal transduction cascades comprising receptors and MAP kinase modules as well as transcription factors mediating ROS (reactive oxygen species) signaling have been characterized.

Very recently, however, we discovered that the ubiquitously distributed CCAAT-binding factor plays a key role in the coordination of oxidative stress response, growth and both sexual and asexual development. This heterotrimeric complex is evolutionary conserved in eukaryotic organisms including fungi, plants and mammals. In *A. nidulans*, the corresponding complex was designated AnCF (*A. nidulans* CCAAT-binding factor) and consists of the subunits HapB, HapC and HapE. All three subunits are necessary for DNA binding. Deletion mutants in either of the subunits showed reduced growth, production of reduced numbers of conidia and were unable to propagate sexually. Hence, AnCF is a central transcriptional regulator of the cell affecting all major differentiation processes. For long, it remained a mystery how the complex is regulated.

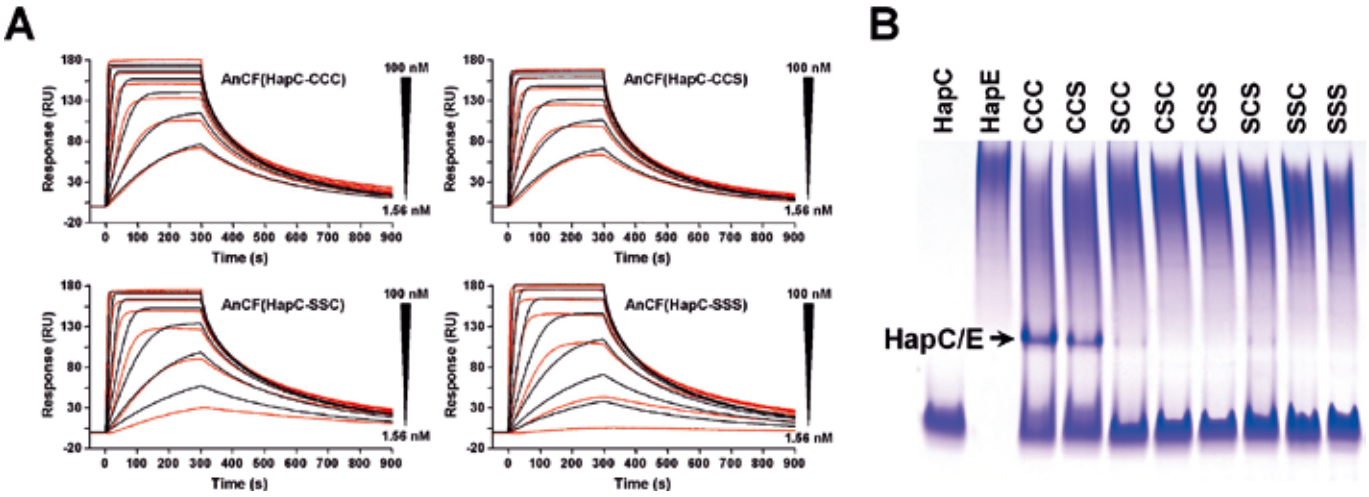

Figure 4
HapC cysteine residues contribute to stability and high affinity DNA binding of AnCF. (A) Surface Plasmon Resonance analysis of AnCF binding to DNA containing a CCAAT box from the *A. nidulans napA* promoter. (B) Native PAGE analysis of HapC/HapE heterodimer formation using all possible HapC cysteine to serine mutants.

The CCAAT-binding complex coordinates the oxidative stress response in *Aspergillus nidulans*

Surprisingly, the core AnCF is regulated via the redox status of the cell mediated by the thioredoxin A system. In detail, we found that the core AnCF senses the redox status of the cell via oxidative modification of thiol groups in HapC. All HapC orthologs from eukaryotes contain three conserved cysteine residues in their core histone fold motif. Oxidized HapC is unable to participate in AnCF assembly and located in the cytoplasm, but can be recycled *in vitro* and *in vivo* by the thioredoxin system, which was characterized by us as well. Mutational and *in vitro* interaction analysis revealed that two of these cysteine residues are indispensable for stable HapC/HapE subcomplex formation and high affinity DNA-binding of AnCF (Figure 4).

By Northern blot analysis we confirmed that several genes/proteins involved in redox regulation and detoxification of ROS like catalase B, thioredoxin, thioredoxin reductase, peroxiredoxin, glutathione peroxidase, glutathione reductase and γ-glutamylcysteine synthetase are activated by the peroxide sensor NapA. Paradoxically, our data revealed that AnCF acts as a repressor of *napA* and in parallel as an indispensable activator of *catB* expression. Therefore, based on our findings about redox regulation of AnCF, cellular ROS accumulation leads to increased cytoplasmic localization of oxidized HapC and consequently, derepression of napA and repression of *catB*. Activation of NapA allows for increased production of thioredoxin and thioredoxin reductase, which are both required for reduction of oxidized HapC. Supported by the known AnCF-mediated auto-regulation of the *hapB* gene, i.e., a mechanism controlling AnCF levels in the nucleus by a piggy-back transport, *hapB* expression is derepressed as well under oxidative stress conditions. As *hapC* and *hapE* are constitutively expressed, increased cellular

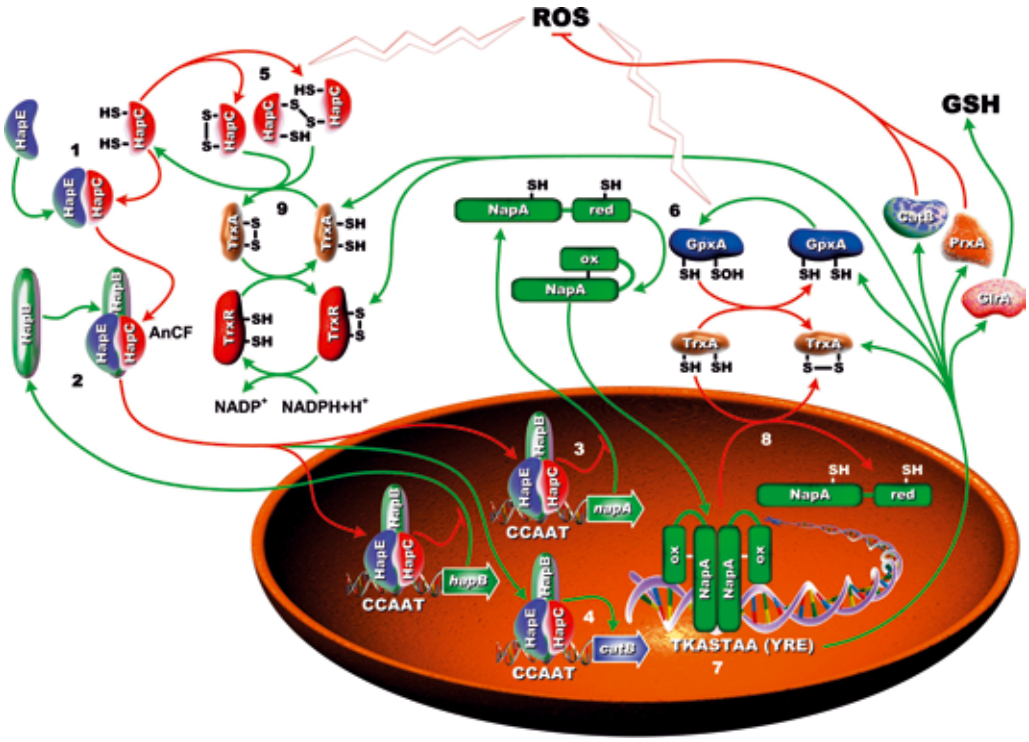

Figure 5
Proposed mechanism for adaptation to oxidative stress in *A. nidulans*. The bold numbers in the figure refer to the following steps. (1) Reduced HapC stabilizes the HapE subunit by interaction through the histone fold motifs localized in each subunit. (2) HapB-mediated piggy back transport of AnCF into the nucleus and AnCF-mediated auto-regulation (repression) of the *hapB* gene. (3) AnCF represses full expression of *napA* and some NapA target genes, but is required for basal expression of *catB*. (5) Reactive oxygen species (ROS) inactivate AnCF via oxidation of HapC and (6) activate NapA by the redox transducer GpxA (glutathione peroxidase-like protein A), leading to (7) increased expression of *napA* and NapA target genes. (8) ROS trigger the activation of the thioredoxin system which leads to inactivation of NapA activity and (9) reactivates AnCF in a feedback loop. This (4) enables production of CatB.

HapB levels result in a higher nuclear AnCF concentration allowing for successive induction of catalase B production (Figure 5).

Taken together, the CCAAT-binding complex is one of the major redox sensors of the cell and a member of a fine-tuned mechanism for the adaptation to different levels of oxidative stress. AnCF and NapA are controlled by interconnected feedback loops. ROS inactivate AnCF via oxidation of HapC, which increases expression of *napA* and NapA target genes directly via release of AnCF repression and indirectly via NapA activation. This response includes the activation of the thioredoxin system, which represses NapA activity and reactivates AnCF. The coordinated activation and deactivation of antioxidative defense mechanisms, i.e., production of enzymes such as catalase, thioredoxin or peroxiredoxin, and maintenance of a distinct glutathione homeostasis very likely represents an evolutionary conserved regulatory feature of the CCAAT-binding complex in eukaryotes.

4 Regulation of fungal secondary metabolism genes

Group Leader: Axel A. Brakhage

Activation of silent gene clusters: Intimate bacterial-fungal interaction triggers biosynthesis of archetypal polyketides in *Aspergillus nidulans*

Fungi produce numerous low molecular weight molecules endowed with a multitude of biological activities. However, mining the full genome sequences of fungi indicates that their potential to produce secondary metabolites is greatly underestimated. Sequence alignments suggest that *A. nidulans* has the potential to generate up to 32 polyketides, 14 nonribosomal peptides and 2 indole alkaloids (in close collaboration with the Research Group Bioinformatics/Systems Biology, Reinhard Guthke). This high number of putative metabolite biosynthesis gene clusters is greater than the known metabolites ascribed to

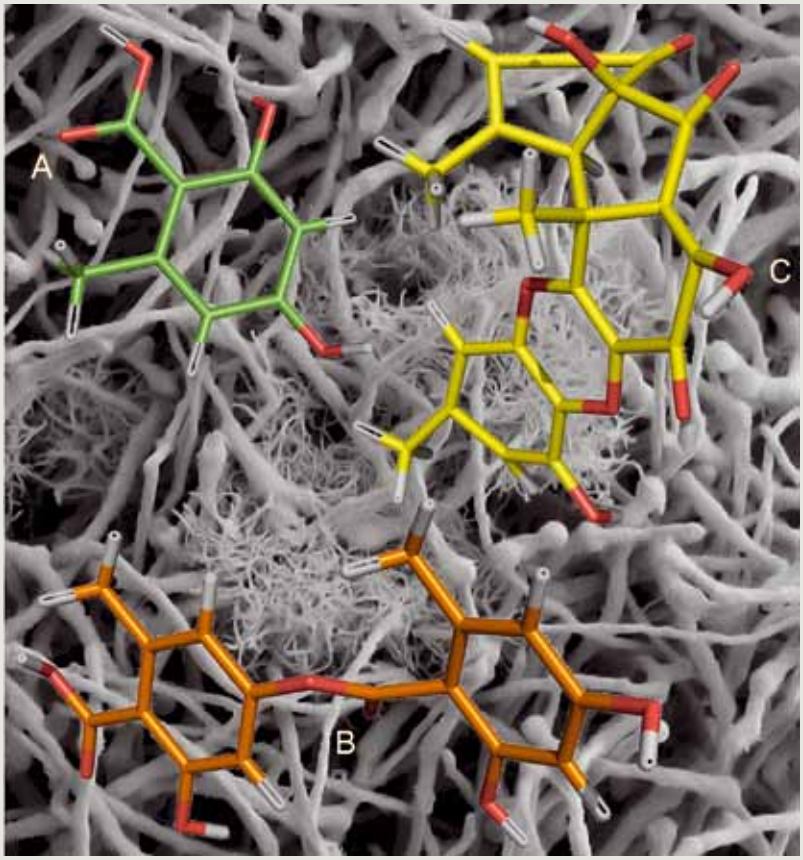

Figure 6
The intimate *trans* domain interaction of *A. nidulans* and *S. rapamycinicus* (background) triggers in the formation of the secondary metabolites orsellinic acid (A), lecanoric acid (B) and F-9775A (C) and F-9775B by the fungus (Schroeckh *et al.*, 2009).

this species, and it may be a reflection of failure of many clusters to be expressed, at least under the culture conditions commonly used in laboratories. Recently, we reported a novel method to activate such silent gene clusters by genetic engineering, i.e., by overexpression of a pathway specific transcription factor which led to the production of so far not identified metabolites in *A. nidulans*.

One of the major challenges in microbiology, however, is to understand the physiological conditions under which these genes are activated. We reasoned that natural products are important for communication between microorganisms. Therefore, to examine the impact of microbial interplay on the expression of silent fungal biosynthesis gene clusters, in close collaboration with the Department of Biomolecular Chemistry (Christian Hertweck) we cocultured *A. nidulans* with a collection of soil-dwelling bacterial strains that share the same habitat. In total, 58 different actinomycetes that included different genera and species were probed. By microarray analyses of both *Aspergillus* secondary metabolism and full-genome arrays, Northern blot and quantitative RT-PCR analyses, we could demonstrate at the molecular level that only a single strain, designated *Streptomyces rapamycinicus* [ATCC 29253], specifically induced fungal biosynthesis genes. According to the microarray data, 2 putative polyketide synthase (PKS, AN7909) and non-ribosomal peptide synthetase (NRPS, AN7884) gene clusters were clearly up-regulated. Most surprisingly, dialysis experiments and electron microscopy indicated that an intimate physical interaction of the bacterial and fungal mycelia is required to elicit the specific response. Such a tight association, as in *S. rapamycinicus* and *A. nidulans*, with a concomitant specific metabolic response is yet unprecedented.

Gene knock out experiments provided evidence that one induced gene cluster codes

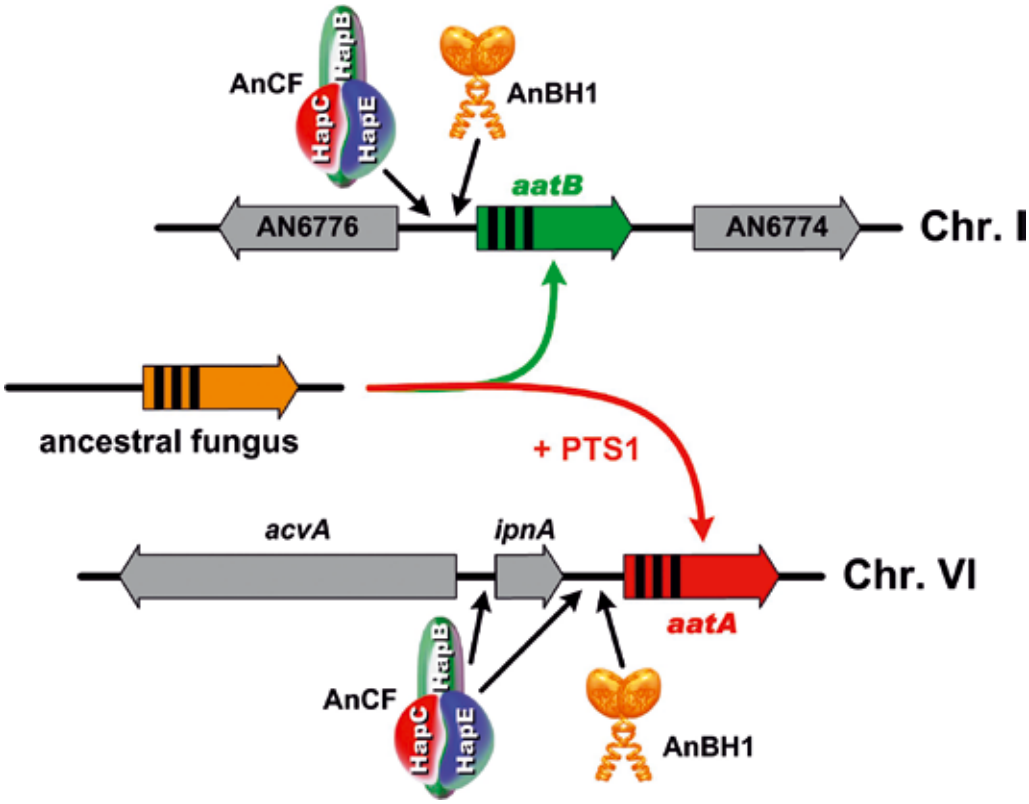

Figure 7
Model of the evolution of the penicillin biosynthesis gene cluster in a putative ancestor of *A. nidulans* (Brakhage *et al.*, 2009).

for the long-sought after PKS required for the biosynthesis of the archetypal polyketide orsellinic acid and the typical lichen metabolite lecanoric acid. Yet, there was only a single report on filamentous fungi producing this ATP synthesis and electron transfer inhibitor. It is intriguing that this compound is usually found in a fungal/bacterial mutualism, and thus likely plays a role in microbial communication. On the other hand, because lecanoric acid inhibits ATP synthesis and electron transfer, it is also conceivable that the bacterium has elicited a fungal defense strategy affecting organisms that are susceptible to lecanoric acid mediated energy breakdown. Because the inducing bacterium is not affected, one may even speculate about a symbiotic fungal bacterial relation. In addition, the two yellow-orange pigments F-9775A and F-9775B are produced as minor compounds. Both compounds were originally isolated from *Paecilomyces carneus* and are inhibitors of cathepsin K, thus damping osteoporosis.

Furthermore, a phylogenetic analysis demonstrates that orthologs of the specifically induced PKS are widespread in nature in all major fungal groups, including mycobionts of lichens.

The obtained results provide evidence of specific interaction among microorganisms belonging to different domains and support the hypothesis that not only diffusible signals but intimate physical interactions contribute to the communication among microorganisms and induction of otherwise silent biosynthesis genes.

Identification of the novel penicillin biosynthesis gene *aatB* of *Aspergillus nidulans* and its putative evolutionary relationship to this fungal secondary metabolism gene cluster

Penicillins and cephalosporins are β-lactam antibiotics. The formation of hydrophobic penicillins has been reported in fungi only, no-

tably *Penicillium chrysogenum* and *Aspergillus (Emericella) nidulans*, whereas the hydrophilic cephalosporins are produced by both fungi, e.g., *Acremonium chrysogenum* (cephalosporin C), and bacteria. The producing bacteria include Gram-negatives and Gram-positives, e.g., *Streptomyces clavuligerus* (cephamycin C) and *Lysobacter lactamgenus* (cephabacins), respectively. The evolutionary origin of ß-lactam biosynthesis genes has been the subject of discussion for many years, and two main hypotheses have been proposed: (i) horizontal gene transfer (HGT) from bacteria to fungi or (ii) vertical decent. There are strong arguments in favour of HGT, e.g. unlike most other fungal genes, β-lactam biosynthesis genes are clustered and some of these genes lack introns. In contrast to *S. clavuligerus*, all regulators of fungal β-lactam biosynthesis genes represent wide-domain regulators that are not part of the gene cluster. If bacterial regulators were co-transferred with the gene cluster from bacteria to fungi, most likely they would have been non-functional in eukaryotes and lost during evolution. Recently, we discovered a novel penicillin biosynthesis gene *aatB*, which is not part of the penicillin biosynthesis gene cluster and is even located on a different chromosome. The aatB gene is regulated by the same regulators AnCF and AnBH1 as the penicillin biosynthesis gene *aatA(penDE)*. Data suggest that *aatA* and *aatB* are paralogues derived by duplication of a common ancestor gene. This data supports a model in which part of the β-lactam biosynthesis gene cluster was transferred to some fungi, i.e. the *acvA* and *ipnA* gene without a regulatory gene. We propose that during the assembly of *aatA* and *acvA-ipnA* into a single gene cluster, recruitment of transcriptional regulators occurred along with acquisition of the duplicated *aatA* ancestor gene and its *cis*-acting sites.

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Axel Brakhage, Volker Schroeckh

Deutsche Forschungsgemeinschaft
Excellence Graduate School Jena School for Microbial Communication JSMC
Teilprojekt: Analysis of the interaction of the human pathogenic fungus *Aspergillus fumigatus* with immune effector cells by functional genomics
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Teilprojekt: Identification of virulence determinants of the human-pathogenic fungus *Aspergillus fumigatus* by proteome analysis
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Teilprojekt: Holistic approach to genomics of human-pathogenic fungi: Data warehouse for integration of data on transcriptome, proteome and metabolome of *Candida albicans* and *Aspergillus fumigatus*
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Bundesministerium für Bildung und Forschung
ERA-NET PathoGenoMics
Die Zellwand als Target zur Verbesserung der antifungalen Therapie der Aspergillose
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European Union
6. Forschungsrahmenprogramm der EU
Specific Targeted Project: Integrating and strenghtening the European Research Area MANASP: Development of novel management strategies for invasive aspergillosis
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European Union
6. Forschungsrahmenprogramm der EU
Marie Curie Research Training Networks
SIGNALPATH: MAP kinase cascades controlling virulence in fungi: from signals to pathogenicity response
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European Union
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Integrated Project: NM4TB: New medicines for tuberculosis
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Freistaat Thüringen
ProExzellenz Initiative
NanoConSens: Nanocontainer und nanostrukturierte Trägermaterialien für Sensorik und Wirkstofftransport
Axel Brakhage, Christian Hertweck

Freistaat Thüringen
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MikroPlex: Analyse mikrobieller Kontaminationen und ihrer Sekundärmetabolite in komplexen Medien
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Freistaat Thüringen
ProExzellenz Initiative
MikroInter: Microbial Interactions
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Selected publications
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Wollbold J, **Guthke R**, Ganter B (2008) Constructing a knowledge base for gene regulatory dynamics by formal concept analysis methods. *Lecture Notes in Computer Sciences* 5147, 230-244.

Ibrahim-Granet O, Dubourdeau M, Latge JP, Ave P, Huerre M, **Brakhage AA**, **Brock M** (2008) Methylcitrate synthase from *Aspergillus fumigatus* is essential for the manifestation of invasive aspergillosis. *Cell Microbiol* 10, 134-148.

Grosse C, **Heinekamp T**, **Kniemeyer O**, **Gehrke A**, **Brakhage AA** (2008) Protein kinase A regulates growth, development and pigmentation in *Aspergillus fumigatus*. *Appl Environ Microbiol* 74, 4923-4933.

Sproete P, Hynes MJ, **Hortschansky P**, **Scharf D**, **Wolke S**, **Brakhage AA** (2008) Evolution of fungal secondary metabolism genes: Characterisation of the novel penicillin biosynthesis gene *aatB* of *Aspergillus nidulans*, the putative ancestor of *aatA*. *Mol Microbiol* 70, 445-461.

Aimanianda V, Bayry J, Bozza S, **Kniemeyer O**, Perruccio K, Elluru SR, Clavaud C, Paris S, **Brakhage AA**, Kaveri SV, Romani L, Latgé JP (2009) Surface hydrophobin prevents immune recognition of airborne fungal spores. *Nature* 460, 1117-1121.

Makarov V*, Manina G*, Mikusova K*, **Möllmann U***, Ryabova O, Saint-Joanis B, Dhar N, Pasca MR, Buroni S, Lucarelli AP, Milano A, De Rossi E, Belanova M, Bobovska A, Dianiskova

P, Kordulakova J, Sala C, Fullam E, Schneider P, McKinney JD, Brodin P, Christophe T, Waddell S, Butcher P, Albrethsen J, Rosenkrands I, Brosch R, Nandi V, Bharath S, Gaonkar S, Shandil RK, Balasubramanian V, Balganesh T, Tyagi S, Grosset J, Riccardi G, Cole ST (2009) Benzothiazinones kill *Mycobacterium tuberculosis* by blocking arabinan synthesis. *Science* 324, 801-804, *equal contribution

Schroeckh V, **Scherlach K**, **Nützmann HW**, **Shelest E**, **Schmidt-Heck W**, **Schuemann J**, **Martin K**, **Hertweck C**, **Brakhage AA** (2009) Intimate bacterial-fungal interaction triggers biosynthesis of archetypal polyketides in *Aspergillus nidulans*. *Proc Natl Acad Sci USA* 106, 14558-1456.

Vödisch M, **Albrecht D**, **Leßing F**, **Schmidt A**, **Winkler R**, **Guthke R**, **Brakhage AA**, **Kniemeyer O** (2009) Two-dimensional proteome reference maps for the human-pathogenic filamentous fungus *Aspergillus fumigatus*. *Proteomics* 9, 1407-1415.

Schmaler-Ripcke J, **Sugareva V**, **Gebhardt P**, **Winkler R**, **Kniemeyer O**, **Heinekamp T**, **Brakhage AA** (2009) Production of pyomelanin, a second type of melanin, via the tyrosine degradation pathway in *Aspergillus fumigatus*. *Appl Environ Microbiol* 75, 493-503.

Thön M, **Al-Abdallah Q**, **Hortschansky P, Scharf DH**, Eisendle M, Haas H, **Brakhage AA** (2010) The CCAAT-binding complex coordinates the oxidative stress response in eukaryotes. *Nucleic Acids Res* 38 1098-1113.

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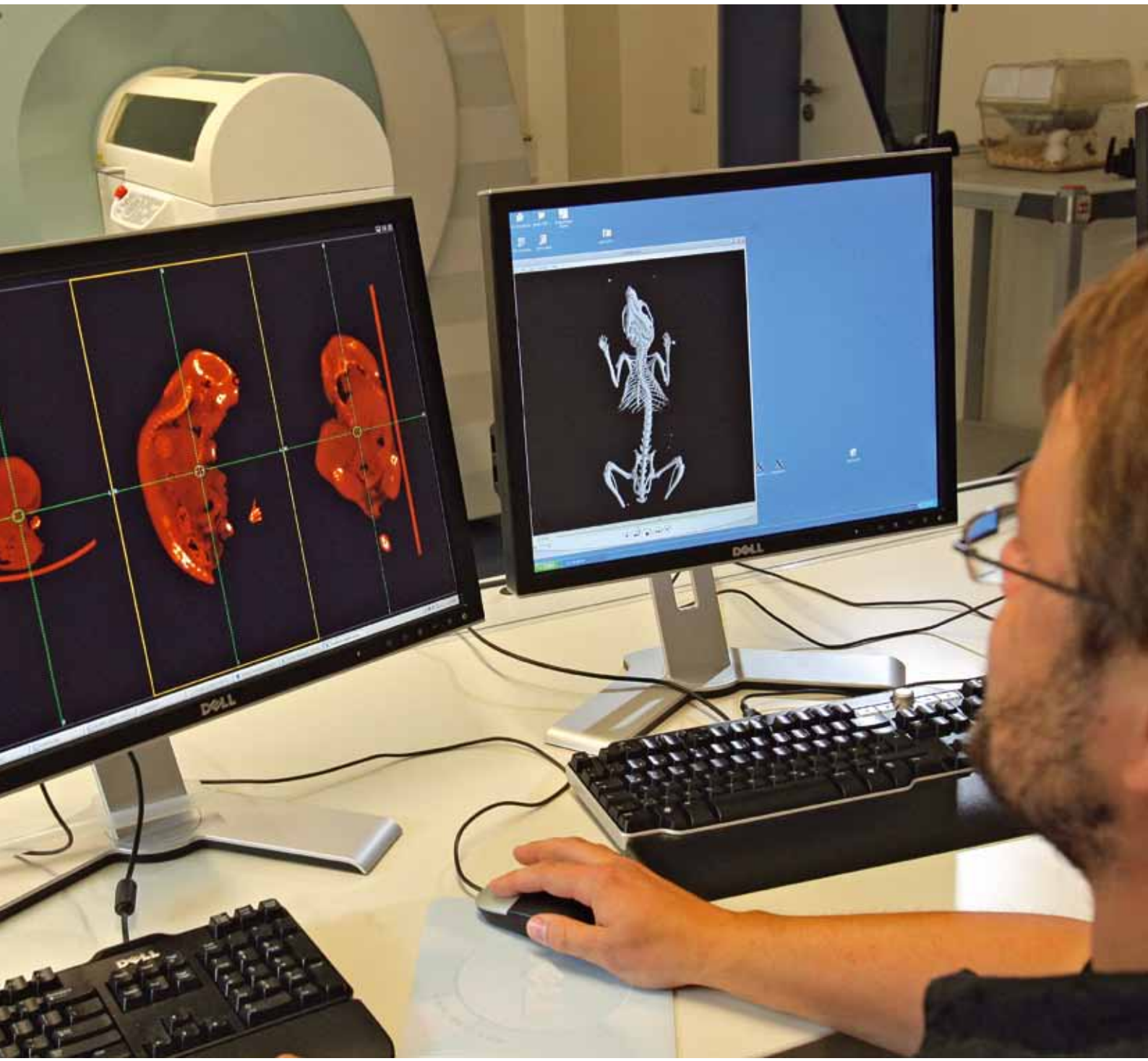

Department of Cell and Molecular Biology

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Research in the Department of Cell and Molecular Biology is devoted to the flow of molecular information during host-pathogen interactions. As model hosts we are using human cell lines, mice and chick embryos *in ovo*, and the pathogens of our main interest are Chlamydiales and pathogenic fungi. To broaden information-solving abilities we have set out to adopt and to develop highly advanced micro- and nanosytems, which allow the simultaneous handling of multiple samples within sets of different biomolecules under nearly identical experimental conditions. At present we are focussing on parallel Rapid PCR, *in vivo* biopolymer-interaction technologies and multicolor hyperspectral imaging of biomolecules on solid body surfaces.
Within the framework of our biological projects, we aim at the elucidation of how infections proceed in living organisms (imaging) and how infected organs react on a molecular level (e.g. comparative transcriptomics and interactomics). Imaging is performed by means of our latest generation positron emission tomography-computed tomography (PET-CT) instrument that provides co-registered images, ie. it combines the high spatial resolution and anatomical detail of CT with the molecular, quantifiable images obtained by PET. Both comparative transcriptomics and interactomics involve massively parallel sequencing.
Micro- and nanosystems, which we have been developing, are also ideally suited for the application in other departments of our institute, as well as within the entire "Beutenberg Campus" in Jena, where research interests in dif-

INTRODUCTION | EINLEITUNG

Head:
Prof. Dr. Hans Peter Saluz

Die Abteilung Zell- und Molekularbiologie beschäftigt sich mit dem Fluss molekularer Information bei Wirt/Pathogen-Interaktionen. Als Modellwirte setzen wir menschliche Zellen, Mäuse und Hühnerembryonen im Ei ein und die Pathogene, die für uns eine zentrale Rolle spielen, sind Chlamydien und pathogene Pilze. Um die verschiedenen, wirtsspezifischen Antworten wirkungsvoll erfassen zu können, entwickeln wir hochmoderne Mikro- und Nano Systeme. 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, *in vivo* Biopolymerinteraktionstechnologien und mit hyperspektralem Vielfarben-Imaging von Biomolekülen auf Festkörperoberflächen.

Im Rahmen der biologischen Projekte erforschen wir Infektionsabläufe in lebenden Organismen (Imaging) und auch wie die infizierten Organe auf molekularer Ebene reagieren (z. B. komparative Transcriptomics und Interactomics). Imaging führen wir vor allem mit einem modernen kombinierten PET-CT (Positron Emission Tomography-Computed Tomography) Instrument durch, was die sehr hohe räumliche Auflösung und anatomischen Details des CT mit molekular quantifizierbaren Bildern des PET kombiniert. Sowohl die Transkriptom- wie auch die Interaktomanalysen involvieren Hochdurchsatzsequenzierung.

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

ferent fields, such 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 expand optimum access to the most advanced scientific know-how and technical equipment we also cooperate with several institutions and industries, locally and internationally. Experience and knowledge gained from our projects allow us to teach and confront students in the university theoretically and practically with modern aspects of basic and applied research.
During the last two years, several diploma and doctoral students have graduated successfully from our department. Within our technological framework, we have had some real success related to rapid heat block thermocycling of small samples. The ability to rapidly amplify nucleic acids has been particularly important for pathogen detection in diagnostic applications, as well as in life science research and industrial applications. Additionally developments concerning *in vivo* ChIPSeq are currently providing novel and unexpected information on regulatory protein-DNA interactions in promoter, intron, and exon regions of the human genome. Finally, our home-made hyperspectral imager coupled with multivariate data analysis provides a powerful new tool for understanding complex biological and the analysis of biomedical samples.

Weise aufeinander treffen. Außerdem liefern diese Systeme, kombiniert mit der Möglichkeit zur Automatisierung, eine Voraussetzung für die effiziente Realisierung von Produkten und Instrumenten, was ein nicht unwesentlicher Aspekt für die „BioRegio“ in Jena ist.

Um eine optimale Erweiterung durch neueste wissenschaftliche Erkenntnisse und technische Mittel zu haben, kooperieren wir mit mehreren lokalen und internationalen Instituten und der Industrie. 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 vertraut zu machen. So betreuten wir in den vergangenen beiden Jahren wiederum mehrere Diplomanden und Doktoranden in unserer Abteilung, am Friedrich-Löffler-Institut und am Max Planck Institut für chemische Ökologie in Jena, die ihre Studien mit Erfolg abschließen konnten.

Was unsere technischen Projekte anbetrifft, konnten wir guten Erfolg in Hinblick auf Rapid PCR verzeichnen. Die Möglichkeit, Nukleinsäuren sehr rasch und präzise amplifizieren zu können, war vor allem für den Nachweis von Pathogenen bei diagnostischen Anwendungen – aber auch für Forschungs- und Industrieanwendungen – wichtig. Andererseits ergaben die Interaktomanalysen neuartige und unerwartete Informationen über die Interaktionen von regulatorischen Proteinen mit DNA in allen Promoter-, Intron- und Exonregionen des menschlichen Genoms. Schließlich ermöglicht das selbstgebaute hyperspektrale Imaging Instrument, kombiniert mit multivariater Datenanalyse, neue Möglichkeiten zum Verständnis von biologischer und biomedizinischer Materialien.

1 Conidia of *Aspergillus fumigatus* inhibit staurosporine-induced apoptosis of alveolar macrophages

Group Leader: Hans Peter Saluz
(cooperation with A. Brakhage, HKI, Jena)

Infections with mold pathogens have emerged as an increasing risk faced by patients under sustained immunosuppression. Species of the *Aspergillus* genus account for most of these infections, and in particular *Aspergillus fumigatus* (Af) can be regarded as the most important airborne pathogenic fungus. The improvement in transplant medicine and the therapy of hematological malignancies are often complicated by the threat of invasive aspergillosis (IA). Specific diagnostics are still limited, as are the possibilities of therapeutic intervention. Hence, IA is still associated with a high mortality rate that ranges from 30 % to 90 %. Recognition of invading microorganisms by the innate immune system is a first and essential step in their successful elimination. Alveolar macrophages are the major resident cells of the lung alveoli; they, along with neutrophils (which are actively recruited during inflammation), are the major cells in the phagocytosis and clearance of Af. Many extra- and intracellular pathogens evolved different mechanisms to escape the innate immune system by directly interfering with apoptotic pathways of phagocytic cells. Unfortunately, there is still little data on the processing of conidia by the host and the effect of conidia on apoptosis of immune cells. Therefore, the aim of this study is to investigate the interaction between Af conidia and alveolar macrophages and the effect on macrophage apoptosis in particular. In previous experiments, we showed that Af conidia inhibit staurosporine induced apoptosis of murine alveolar macrophages in a caspase-3-dependent manner. In order to elucidate the molecular mechanism of this anti-apoptotic effect, proteomic (two-dimensional gel electrophoresis) and transcriptomic (Microarray and Digital Gene Expression Profiling) approaches are performed.

2 Two-laser, large field hyperspectral microarray scanner for the analysis of multicolour microarrays

Group Leader: Hans Peter Saluz
(cooperation with GMBU, Jena)

As part of a comprehensive multicolour genotyping microarray research program, we have developed in cooperation with GMBU (Jena) a two-laser large field hyperspectral multicolour microarray scanning system (Erfurth *et al.*, 2008). In contrast to other systems, the hyperspectral microarray scanner detects mixed signals from several dyes at once and then unmixes the signals by computational means, thus eliminating the need for fixed narrow band optical filters for each dye. Hyperspectral microarray scanners potentially enable the use of a broad range of spectrally overlapping dyes. Our system was designed to enable the simple exchange of lasers covering different spectral regions. We tested it for a wide range of different glass substrates, fluorescent labels, and imaging conditions, such as dry surfaces and PBS/glycerol aqueous environments.

Of the multiple existing methods for genotyping, those based on the application of microarray technology offer the greatest potential for cost-effective analysis of many genetic markers at one time. Fluorescence-based single-base extension (SBE), sometimes referred as minisequencing, is used in a broad range of microarray formats ranging from ultra-high density bead arrays to in-house spotted focussed microarrays oriented toward the analysis of a limited number of mutations. Small focussed arrays are finding direct application

References
Erfurth F et al. (2008) *Anal Chem* 80, 7706–7713.

in the diagnosis of genetic diseases, genotyping of viruses, genotyping of bacteria, genotyping of fungi, and in forensic applications. These arrays are produced by the spotting of short oligonucleotides on activated substrate surfaces in formats such as individual arrays, "array-of-arrays", or in 96-well and 384-well glass bottom microplates. Alternatively, universal high-density tag arrays containing thousands of anti-tag probes can be produced by *in situ* synthesis. These assays, which generally utilize between one and four fluorescently labelled dideoxy terminators (ddNTPs) for performing SBE reactions and various commercial scanners containing up to four lasers, have been described in the context of genotyping via microarray. A four-colour labelling strategy maximizes the multiplexing ability of SBE reactions, thereby allowing all possible allele combinations to be analysed simultaneously. This strategy has been applied in SNaPshot assays using subsequent size separation via capillary gel electrophoresis, in arrayed primer extension (APEX), and in spotted tag arrays generated in house. SNaPShot assays use terminators labelled by four dichlororhodamine dyes. The dyes are separated from conjugation to ddNTPs by ethylene oxide linkers, which improves nucleotide incorporation efficiency by polymerases. In addition, they have narrow spectral emission bandwidths that improve their spectral separation by capillary electrophoresis systems equipped with a spectrograph and a cooled CCD camera. They occupy, however, a visible spectral range from 540 nm to 615 nm, and their spectra are separated by only 25 nm to 30 nm. On the other hand, a commercial confocal four-laser microarray scanner described by other authors is equipped with a blue argon (488 nm) laser, a green HeNe (544 nm) laser, a yellow HeNe (594 nm) laser and a red HeNe (633 nm) laser, and the corresponding narrow bandpass filters. It is clear that the scanner cannot optimally separate dichlororhodamine dyes because of their 25 nm to 30 nm spectral separation; such a device would require dye sets with a spectral separation of approximately 50 nm. In addition, the excitation spectra of d-rhodamine dyes do not match the lasers in the scanner described. In recent years, numerous terminators labelled with infrared cyanine dyes were developed for capillary electrophoresis sequencing, e.g. WellRED dyes from Beckman. It was demonstrated that these dyes can be efficiently excited by 635 nm, 675 nm, 750 nm and 810 nm lasers in capillary electrophoresis sequencing applications. The use of these novel dye-labelled terminators in multicolour genotyping experiments has been impossible due to the lack of appropriate scanners working in the infrared spectral region; poor sensitivity of the photomultipliers (PMT) in infrared has been the chief barrier to implementation. Therefore, the performance of infrared dyes in the context of microarrays has never been fully evaluated. Unfortunately, some new four-dye sets of labelled terminators are commercially available only in mixtures with unlabelled dNTPs which prevent their use in SBE-minisequencing reactions. The present strategy of testing various commercially available dye-ddNTP combinations in four-colour microarray genotyping is mainly predicated upon finding dyes that will match the optical parameters of commercial four-laser scanners rather than upon characteristics such as incorporation efficiency of labelled ddNTPs, chemical and photostability of the dyes, quantum yield, etc. Actually, in practice it is impossible to find commercially available sets of four dyes that do not show optical crosstalk between the channels. This problem invariably results in complicated numerical signal corrections being required downstream following their use. At present, four-dye sets used in microarray genotyp-

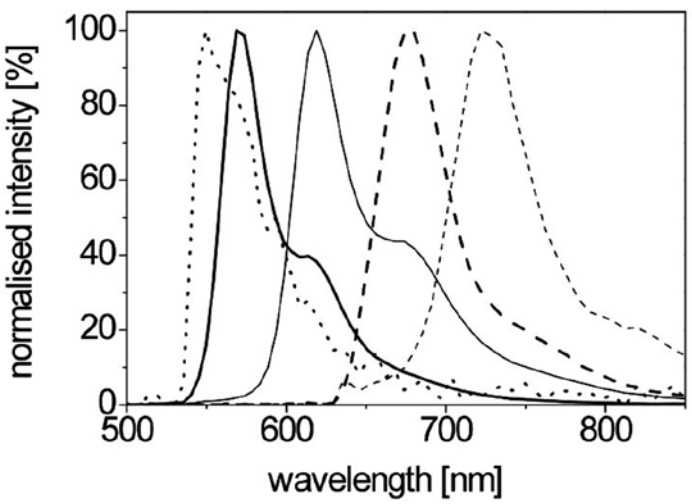

Figure 1
Spectral signatures of single fluorescent sources on a 4-dye-labelled microarray: Cy3 (bold solid), Cy3.5 (thin solid), Cy5 (bold dashed), Cy5.5 (thin dashed), and spot-localised contamination (bold dotted).

References

Erfurth F et al. (2008) *Anal Chem* 80, 7706–7713.

Sell S et al. (2008) *Mycoses* 51, 426–427.

ing usually contain mixtures of chemically and structurally different dyes, such as fluoresceins, rhodamines and cyanines (Erfurth *et al.*, 2008). Different dyes require different scanning environments, however. For example, it was shown that fluorescein demonstrates considerable fluorescence quenching and non-linear response on dry microarray surfaces. The quantum yield of Cy3 is dependent on viscosity of microenvironment, and Cy5 is sensitive to ozone and can change spectral properties depending on the microarray substrate used. The aforementioned differences in dye performance can affect the accuracy of four-colour genotyping microarrays. In addition, considerable differences in incorporation efficiency of labelled ddNTPs are considered to be another source of variability in four-colour microarray genotyping experiments. Currently, the four-colour genotyping microarrays have been produced by in-house contact spotting frequently exhibit poor spot morphology, irregular contours, non-uniform pixel signal intensity (donut-shaped spots), etc. Generally, such artefacts need to be removed from analysis, e.g. by sophisticated image-analysis algorithms. It has been reported that poor-quality genotyping arrays demonstrating non-uniform spots yielded less robust genotyping results. Another source of variability concerns non-linear signal intensities due to dye/scanner response. Signal-dependent non-linear bias between channels of cDNA gene expression arrays is corrected by non-linear normalization methods. These methods are based on the assumption that the majority of genes are not differentially regulated. It is clear that these methods cannot be applied to four-colour microarray genotyping experiments. All methods of data analysis in four-colour microarray genotyping experiments use linear normalization thus introducing an additional source of variability due to dye bias.

3 Rapid heat block thermocycling of small samples: Diagnostics of microbial, viral and fungal pathogens in humans and marine organisms

Group Leader: Alexander Tretiakov

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, that 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. The cycler and corresponding consumables have been commercialized by Analytik Jena AG and are commercially available. Based on the above instrument, we established rapid and sensitive 1-step singleplex and multiplex PCR-based diagnostics for the detection of microbial, viral, and fungal pathogens in humans and marine organisms (Sell *et al.*, 2008). The main difference between this thermocycler and conventional instruments is that it rapidly and accurately changes and holds the temperature of small volume reaction mixtures during thermocycling. For example, it cools a reaction mixture between 95° C and 55° C in 14 seconds only (Tretiakov and Saluz, US Pat 6,556,940), while conventional thermocyclers need approximately 40 seconds for 50 µl reactions. The advantage of rapid cooling of reaction mixtures during PCR cycling are well known, i.e. rapid cooling of the reaction mixtures from 95° C to 55° C and short holds at 55° C minimize mis-priming and reduces the generation of unspecific products. In addition, rapid cooling prevents rehybridization of PCR products that causes the so called “plateau effect” in conventional PCR reactions. Rehybridization limits the accumulation of spe-

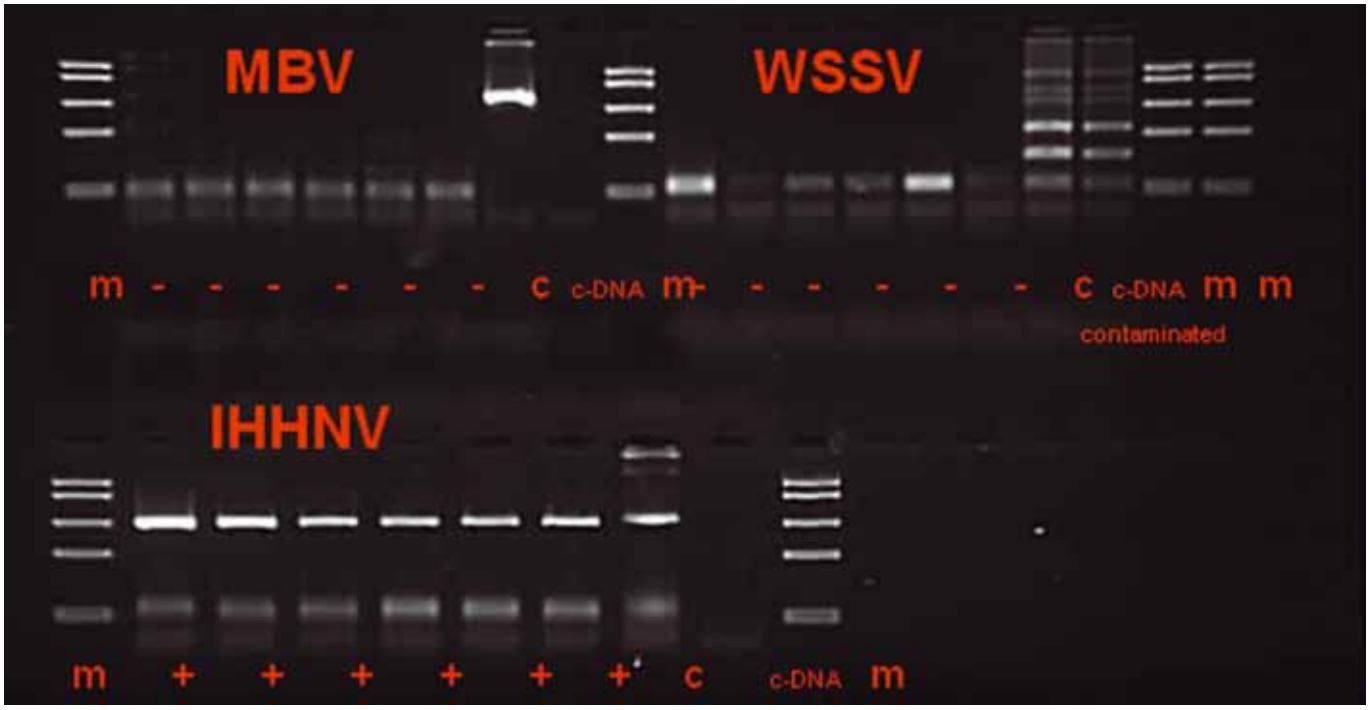

Figure 2
cDNA diagnostics of crustacean pathogens (MBV, WSSV, IHHNV).
+ and – stands for pathogen positive and negative samples, respectively.
m: size markers; c: control

cific PCR products during conventional PCR reactions and reduces the total product yield. Rapid cycle PCR accumulates much more product per 1µl of reaction than conventional PCR. Finally, we combined the above unique properties of rapid cycle PCR with new ultra-fast and ultra-sensitive methods for separation and detection of PCR products in agarose gels in order to develop a 1-step PCR-based technique capable to detect less than 3 viral copies per reaction in 30-40 minutes. We evaluated our new technique by using purified shrimp DNA samples and crude DNA extracts. In addition, we developed a rapid and efficient method for crude DNA extract preparation using the latest generation shaker bead mill from PeqLab.

4 Fabrication and evaluation of a sequence-specific oligonucleotide miniarray for molecular genotyping

Group Leader: Hans Peter Saluz
(cooperation with Javeed Iqbal, Omaha, USA, and Matthias Dürst, Jena, Germany)

Epidemiological studies have increasingly shown a strong association of human papillomaviruses (HPVs) with cervical cancers and benign lesions. Genital HPV infections appear to be the most common sexually transmitted disease, and a majority of them are induced by two HPV types: 6 and 11. In addition, a group of at least 15-18 HPV subtypes (e.g. HPV 16, 18, 31, 33 and 45) are detected in about 80 % of all cervical cancers and most precancerous lesions (cervical intraepithelial neoplasias, CIN 2/3). However, the Pap smear test or hybrid capture II assay (HCA-II), which is the common screening program for HPV-related infection, is limited in its ability by the number of

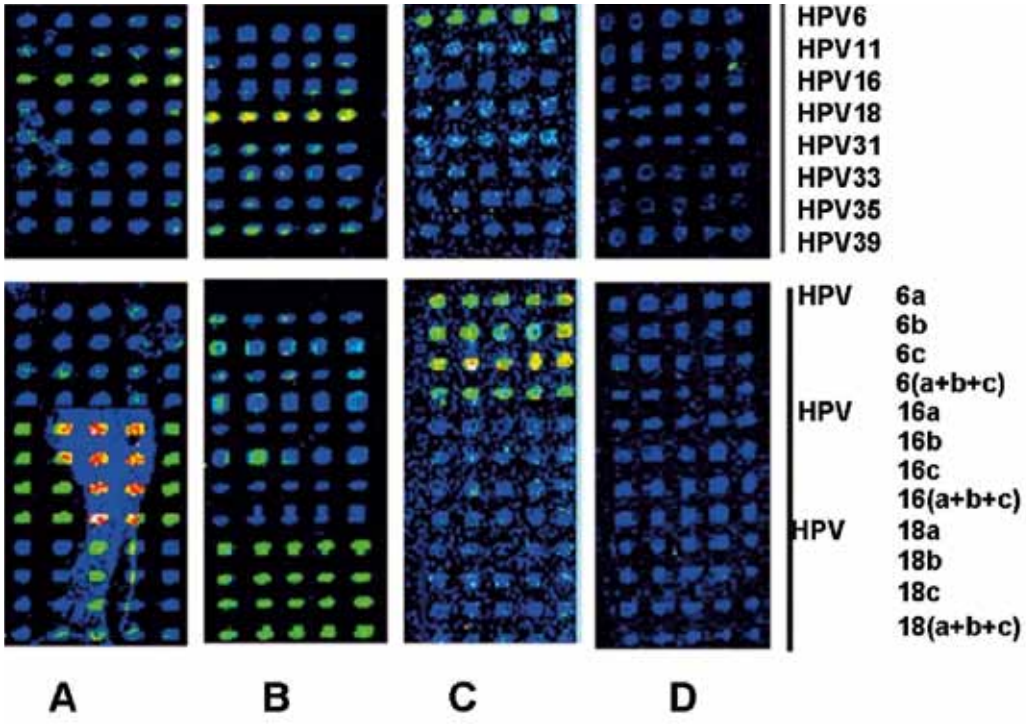

Figure 3
Hybridization pattern with HPV 30-mer oligomers containing 15-mers as confirmatory markers. Probes generated from (A) cloned LI ORF HPV 16 (B) HeLa cell line and (C) HPV 6-positive clinical sample (D) MS 751 cell line as negative control, confirm the specificity of hybridization.

References
Iqbal J et al. (2008) *Indian J Med Microbiol* 26, 13–20.

identified subtypes. HPV subtype detection requires the amplification of the E1 or L1 open reading frame (ORF) of the genome and gene identification by sequencing, restriction endonuclease digestion, or by hybridization using sequence-specific radiolabeled probes.

Recently, the DNA chip assay has been introduced in HPV detection using different DNA microarray platforms, including a few commercial assays e.g. PapilloCheck (Greiner Bio-One, Germany), PreTect HPV proofer (NorChip; Norway), and *HPVDNAChip* (Biomedlab Co., South Korea). To substantiate and further extend the investigation, we studied some of the technical and specificity and sensitivity issues of DNA oligo-array for genotyping. Recognizing the potential of the DNA microarray technology, we studied the fundamental features of this technology and evaluated its significance in molecular subtyping of HPV (Iqbal *et al.*, 2008). The fundamental principle involves the highly selective nature of the complementary DNA double-helix formation and therefore provides a precise analytical power. In our study, the DNA microchip assay was optimized to detect a single mismatch, and various specificity and sensitivity issues were addressed in miniaturized conditions. To assess the application of this simplified assay, the procedure was also employed for HPV genotyping using a 149 basepair (bp) region of L1 ORF from different cell lines and clinical specimens. The HPV subtypes were identified by hybridization with a fabricated oligonucleotide array immobilized with DNA sequence specific for different HPV subtypes. This simplified procedure can be easily adapted for the identification of other pathogens with high sensitivity and specificity a reasonably low cost.

The DNA chips have chemically active groups, such as aldehyde, poly L-lysine, epoxy, or silane deposited on a non porous glass surface on which DNA molecules are immobilized. Different substrate chemistries are the focus, and

methods are being developed for efficient binding of oligonucleotides to different kinds of substrate. In our studies, the aldehyde reactive surface showed maximum binding capacity for amino linked oligonucleotides. In addition, the use of $dT_{(15)}$ spacer overcomes the steric hindrance of the surface structure and solvent layer due to its charge, hydrophobicity, and degree of solvation. The composition of spotting solution, humidity, and hybridization temperature also play an essential and significant role in optimal performance of a microarray assay.

The specificity of a single mismatch discrimination in microarray assay provides significant evidence for using this procedure for HPV genotyping. In our mini array, eight HPV genotypes were selected. They represent major HPV subtypes prevalent globally, including low-risk types of HPV (6 and 11); intermediate risk (33, 35 and 39) and high-risk types (16, 18 and 31). The broad spectrum of HPV genotypes by use of $GP5^{+}/GP6^{+}$ primer set for amplification, and combined sequence specificity of microarray assay makes this attractive tool for diagnostic procedures. However, the origin of the labeled probe remains a critical criterion for specificity. The probes generated from cloned genes or cell lines provide specific signal to noise ratio, whereas the probes from the pathological samples may results in cross hybridization. However, oligonucleotides with high diversity in their sequences can essentially overcome cross hybridization in genotyping assays as shown in HPV microarray.

In our HPV mini array we were able to detect specific genotypes with high accuracy, by both a 15-mer and a 30-mer target oligonucleotide, therefore demonstrating the potential of this technology for broad diagnostic procedures.

In conclusion, our study addressed fabrication and sensitivity issues of targeted DNA array assay and further improvements need to be implemented for the detection of other pathogens and make the use of microarray screening programs more feasible in diagnostic laboratories.

5 Protein-protein interaction networks during infection and cellular stress

Group Leader: Frank Hänel (cooperation with Frank Grosse, FLI, Jena, Germany)

Interactions of typeIII-secreted chlamydial proteins with host proteins

Chlamydiae are obligate intracellular bacterial pathogens that infect a broad range of cell types, including those of the eye, lung and genital tract epithelia. Several species, particularly *Chlamydophila (Cp.) psittaci* and *Cp. abortus*, are transmissible from animals to humans, causing relevant zoonotic infections. The most important animal chlamydiosis of zoonotic character is psittacosis, a systemic disease in psittacine birds of acute, protracted, chronic, or subclinical manifestation. *Chlamydiaceae* develop in a host cell within a membranbound compartment, termed an inclusion, that does not fuse with lysosomes. The membrane of the inclusion is initially formed by the invagination of the plasma membrane and pinching off of a vesicle containing the infectious form of the bacterium, the elementary body (EB). Thereafter, EBs differentiate into noninfectious but metabolically active reticulate bodies (RB), which proliferate within the expanding inclusion, giving rise to 1000 or more progeny per host cell. The infectious cycle ends after 2-3 days depending on the strain, when bacteria that have differentiated back to EBs are released in the extracellular medium. During this unique biphasic developmental cycle replicating bacteria acquire energy and biosynthetic precursors from the infected cell. Furthermore, during the infectious cycle, *Chlamydiaceae* modulate cellular functions such as apoptotic programs and immune response (Ying *et al.*,2007). Studies with inhibitors of bacterial protein synthesis suggest that the modulation of the host cellular functions requires the activity of chlamydial proteins. All *Chlamydiaceae* code for the core components of a Type III Secretion (TTS) apparatus, a protein transport system used by Gram-negative bacteria to translocate proteins into the cytoplasm of the host cell. Therefore, it is commonly accepted that

References

Betts HJ et al. (2008)
Curr Opin Micobiol 11, 1–7.

Ying S et al. (2007)
Curr Immunol Rev 3, 31–40.

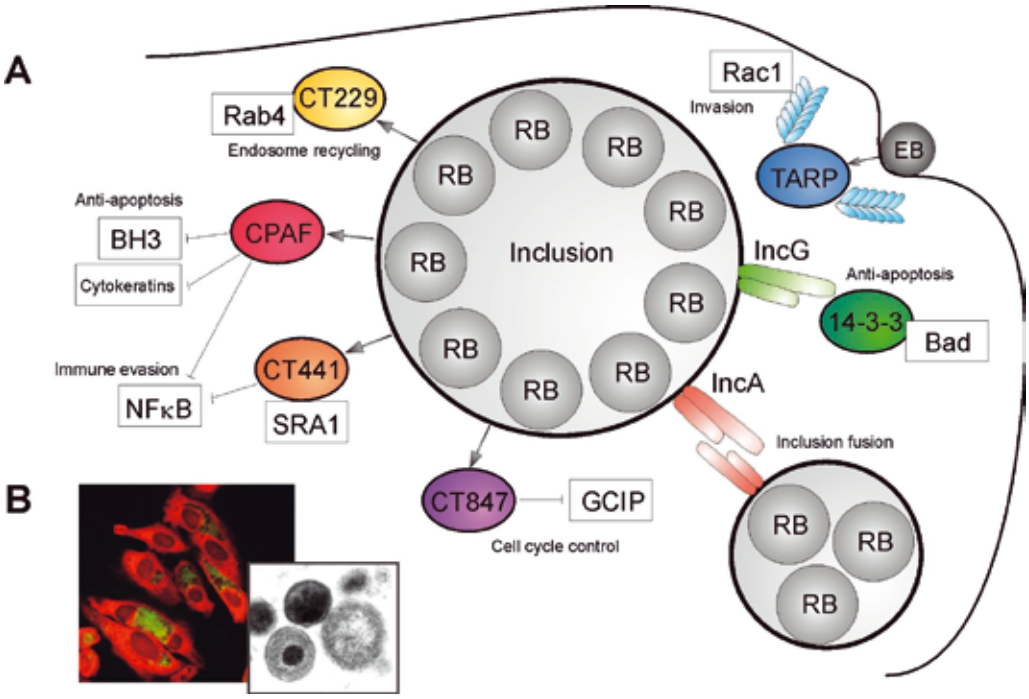

Figure 4

A) Overview of the current known interactions between secreted chlamydial effector proteins and their eukaryotic targets. For *Chlamydia*, as intercellular pathogens, these protein-protein interactions are crucial for processes like endocytosis, immune evasion, or nutrient acquisition.

B) In the host cell *Chlamydia* are encased in a compartment called inclusion (left picture, stained green). During the infection cycle Chlamydia occur in different forms. In a TEM photograph, they can be distinguished through their electron density (right picture). Elementary bodies (infectious form) are more dense than reticular bodies (metabolic active forms) and intermediate bodies.

chlamydial effector proteins are targeted by the TTS to the inclusion membrane (Figure 4). The ability of these microorganisms to express and secrete host-interactive proteins capable of directly modulating relevant eukaryotic pathways represents an important adaptation strategy. Despite our current inability to genetically manipulate *Chlamydiaceae*, characterization of these effector proteins enables chlamydiologists to elucidate molecular mechanisms essential for chlamydial pathogenesis (Betts *et al.*, 2008).

Using the yeast two-hybrid technology, well established in my group, we utilized a selection of secreted chlamydial proteins from zoonotic and human-pathogenic *Chlamydiaceae* as baits in a spate of interaction screens against a human HeLa cDNA library pretransformed into yeast. In this way, we identified a series of host proteins specifically interacting with the chlamydial bait proteins in the yeast system. Selected protein interaction pairs were the subject of continuative biochemical, physiological and cell biological investigations. One example of a more in-depth characterized protein-protein interaction is the interplay of the protease CT441 of *Chlamydia trachomatis* with a co-activator of the human estrogen receptor α (ERα). Using the PDZ domain of the protease CT441 of *Chlamydia trachomatis* as a bait in a yeast two-hybrid screen, we identified the SRAP1 co-activator of ERα as an interacting protein. SRAP1 is a unique modulator of steroid receptor activity, as it is able to mediate its co-regulatory effects both as an RNA and a protein. GST pull-down experiments confirmed the interaction of CT441 and SRAP1 *in vitro*. Furthermore, it was shown that the CT441-PDZ domain fused to a nuclear localization signal was able to bind and to target SRAP1 to the nucleus in mammalian cells. CT441 did not cleave SRAP1, but retained the protein in the cytoplasm and thereby partially alleviated its co-activation of ERα in a heterologous yeast system and in

mammalian cells. This result suggests a new possibility of host control by *Chlamydiaceae*. Chlamydial attachment and infectivity *in vitro* as well as ascending disease and sequelae *in vivo* are enhanced or modulated by estrogen. Furthermore, products of many ERα target genes involved in signal transduction, cell proliferation, and apoptosis may have an influence on chlamydial survival and development in the host. Therefore, it seems plausible that chlamydiae regulate host metabolism by targeting ERα activity. Moreover, the property of CT441-PDZ domain to specifically sequester SRA1 protein but not SRA1 RNA may be used to distinguish between the cellular functions of the SRA1 RNA and protein. This property has clinical relevance as it has been proposed that disturbance of the balance between SRAP1-coding and non-coding SRA1 RNAs in breast tumor tissues might be involved in breast tumori genesis.

Interaction network of the multifunctional signal protein TopBP1

TopBP1 was initially identified as a DNA topoisomerase IIβ-interacting protein. Human TopBP1 possesses eight BRCA1 carboxyl-terminal (BRCT) domains, a motif which was first described at the C- terminus of the breast cancer susceptibility gene product, BRCA1, and which is conserved in many proteins related to the cell cycle checkpoint and DNA damage response. TopBP1 shares sequence homology with *Saccharomyces cerevisiae* Dbp11, *Schizosaccharomyces pombe* Rad4/Cut5, *Drosophila melanogaster* Mutagen-sensitive-101 (Mus101), and *Xenopus laevis* Xmus 101/Cut5. Like its yeast counterparts, human TopBP1 is required for cell survival, DNA replication, resistance to DNA damage and checkpoint control. The literature on TopBP1 also suggests a function as a transcriptional regulator. In response to ionizing radiation, TopBP1 is phosphorylated by the ataxia-telangiectasia mutated kinase (ATM), implying a role of TopBP1 in the DNA damage checkpoint control. This role was directly demonstrated by a later study using TopBP1 antisense oligonucleotides, showing that ionizing radiation-induced G2/M checkpoint and checkpoint kinase 1 (Chk1) phosphorylation was partially abrogated in the absence of TopBP1. Moreover, very recently it was shown that TopBP1 physically interacts with the ATM and Rad3-related (ATR) gene product and greatly enhances ATR's kinase activity (Garcia *et al.*, 2005).

The work of our group on TopBP1 in the previous period was focused on the search and the characterization of new interacting proteins. We performed co-immunoprecipitations with anti-TopBP1 antibodies in different cell lines and analyzed the interacting proteins by a proteomic approach. The MALDI-TOF-TOF experiments were performed in cooperation with the Grosse Group at Fritz Lipmann Institute, Jena. Among others we found a pair of interacting proteins known to be involved both in transcriptional co-repression and in DNA repair. The functional investigation of the interaction between TopBP1 and these proteins are still under way.

In cooperation with J. N. Mark Glover from the University of Alberta structural and functional studies on the sixth BRCT domain of TopBP1 were performed. This BRCT domain of TopBP1 has been implicated in binding to the phosphorylated transcription factor, E2F1, and poly(ADP-ribose) polymerase 1 (PARP1), where the latter interaction is responsible for the poly(ADP-ribosyl)ation of TopBP1 (Wollmann *et al.*, 2007). To gain a better understanding of TopBP1 BRCT6 in phosphopeptide binding and PAR binding, the Glover group solved the crystal structure of TopBP1 BRCT6. The structure shows a canonical BRCT fold with a degenerate phospho-Ser binding pocket. TopBP1 BRCT6 also lacks the hydrophobic surface that is required for packing of two tandem repeats. Taken together, it is unlikely that TopBP1 BRCT6 binds to a phosphopeptide like BRCA1 or MDC1. The structure also provides insight into a potential surface for PAR binding on TopBP1 BRCT6 as well as Glu residues that may be poly(ADP-ribosyl)ated by PARP-1.

References

Garcia V et al. (2005)
DNA Repair 4, 1227–1239.

Wollmann Y et al. (2007)
J Cell Biochem 102, 171–182.

6 Cumulative effect of mutations of the constitutive ABC-transporter gene *bmrA* on resistance to cervimycin C in *Bacillus subtilis*

Group Leader: Hans Krügel (cooperation with Sabine Brantl, Jena, Germany)

In addition to their medical value, many antibiotics are important tools for elucidating the biology of microorganisms. Cervimycin C belongs to a complex of compounds produced by *Streptomyces tendae* and consists of a tetracyclic polyketide decorated with two unusual trideoxysugar chains. This compound is solely active against Gram-positive bacteria, such as *Bacillus subtilis* ATCC 6633, but also against various pathogens (Herold *et al.*, 2005).

Generally, microorganisms are able to adapt to antibiotic stress by a variety of specific and unspecific mechanisms. A more general mechanism affecting hydrophobic drugs is exerted by exporters lowering intracellular drug concentrations either by acting as antiporters or by ATP hydrolysis-driven export (Kerr *et al.*, 2005). For analysis of the genetic changes associated with severe phenotypes, whole genome sequencing can be applied.

Here we report the molecular basis for a mechanism circumventing the action of the new antibiotic cervimycin C on B. *subtilis*. To elucidate the biochemical nature of the CmC resistance observed after propagation of *B. subtilis* at increasing CmC concentrations, genomic sequencing of two resistant mutants and the reference strain were performed. In both mutants, cumulative effects of multiple mutations of a constitutively expressed ABC-transporter conferring resistance to CmC were analyzed.

References

Herold K et al. (2005) *Chemistry* 11, 5523–5530.

Kerr ID et al. (2005) *Biochem Soc Transact* 33, 1000–1002.

7 Positron emission tomography/computed tomography (PET/CT) for depicting infection and inflammation: reduction, refinement, and replacement of animal experiments.

Group Leader: Thomas Opfermann (cooperation with Friedrich-Loeffler-Institute, Jena, and Friedrich Schiller University Jena).

The project includes three sub-projects in the areas of bacterial infections (*Chlamydophila psittaci*), fungal infections (*Candida albicans* and *Aspergillus fumigatus*) and G6PI induced murine arthritis.

Technique and informatics

The Siemens Inveon μPET/CT scanner is from the newest generation of its kind. The scanner was installed in December 2007 at the HKI and subjected to extensive testing. In all subprojects, the first experimental series were carried out using the device, thus gradually integrating it into existing test procedures and models. Measurements to determine the key performance parameters were performed and analyzed. Conditions for specific study subjects (eggs, mice, *ex vivo* samples) have been developed, implemented, and optimized.

Bioinformatic research of the PET/CT workgroup has been focused on the evaluation and implementation of suitable data management applications to cope with the continuously growing amount of PET/CT image data generated in the lab. In addition, semi-automated image analysis and data mining tools are under development to support the PI in a fast, efficient and reproducible data analysis.

Radiochemistry

The radionuclide laboratory was equipped with a custom made glove box appropriate for ^{18}F-fluoride organic chemistry, housed in a glove box for remote controlled multi-step synthesis. Additionally, the lab was equipped with a computer controlled device for ^{68}Ga-gallium labeling chemistry and an analytical radio-HPLC system.

Figure 5
Inveon µPET/CT Scanner at the HKI

Specific tracers to distinguish between inflammation and infection foci would be of great benefit. Therefore, ^{18}F-fluorine-containing intermediates were synthesized using the computer controlled hotbox-3 synthesis module (Scintomics GmbH, Fürstenfeldbruck). Currently, different molecules with high affinity to the pathogens are evaluated as precursors for labeling with ^{18}F-fluoride.

For the detection of foci in G6PI induced murine arthritis and chlamydial infections, ^{68}Ga-gallium and ^{89}Zr-zirconium labeling procedures were adapted for small quantities of antibodies. The first results showed a specific binding of the ^{89}Zr-labeled antibody. $^{89}Zr\text{-}ZrCl_4$ was supplied from FZD Rossendorf, Institute of Radiopharmacy.

For use of a low activity ^{68}Ge/^{68}Ga generator in small animal PET experiments a computer controlled procedure for concentration of ^{68}Ga activity was developed (manuscript in press).

Small animal PET/CT

The anesthetic procedure for mice has been established. A method for anesthesia of chicken embryos *in ovo* was developed resulting in CT and PET images without interfering motion artifacts (manuscript submitted). Another precondition for the measurement in the PET/CT is the appropriate application of the tracer. Two routes have been established. The first possibility is to apply the respective tracer directly on the CAM (Chorioallantoic membrane) and the second method is to apply the tracer intravascular. Comparative tests of both methods with different tracers pointed to different kinetics of accumulation in the embryo.

Until now, commercial tracers (^{18}F-FDG and ^{18}F-fluoride) have been used for initial investigations. In preliminary tests we could demonstrate that ^{18}F-FDG is generally suitable for *in vivo* detection of fungal infections in the egg model.

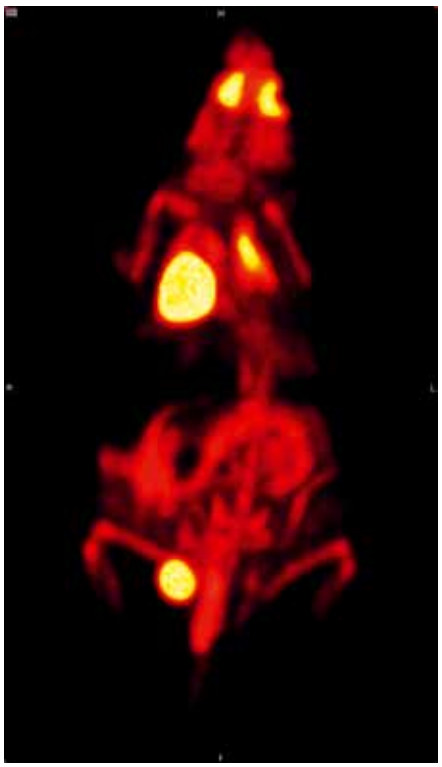

Figure 6
PET image of a *Aspergillus fumigatus* infected mouse

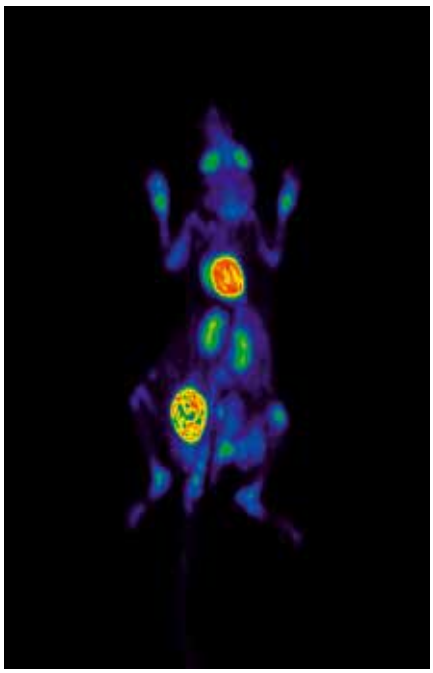

Figure 7
PET image of a mouse with arthritic joints

Infection models of human-pathogenic fungi

In collaboration with the Department of Microbial Pathogenicity Mechanisms (MPM) models of aspergillosis and candidiosis in mice and eggs were studied using PET/CT technique. Animals were administered sublethal doses of pathogens. In ^{18}F-FDG measurements correlation between biochemical analysis and specific uptake values showed a significantly higher uptake in infectious foci (for details see Dept. of MPM).

Embryonated eggs as novel infection model for *Chlamydophila psittaci*

The aim of this project was to establish embryonated chicken eggs as a model system for the investigation of *Chlamydophila psittaci* infections. Therefore, studies on the accumulation and distribution of chlamydial elementary bodies were performed. Based on these data the course of infection, shown by means of molecular biology (i.e. qRT-PCR) should now also be characterized using molecular imaging techniques together with ^{18}F-FDG and another novel ^{18}F-labeled tracer. In competition for the 2010 Siemens award "Image of the Year" we won the place of honorable mention ("Time Resolved Tracer Distribution in an Embryonated Chicken Egg").

PET/CT in experimental arthritis

The purpose of this subproject was to validate usability of PET/CT scanning in experimental G6PI-induced murine arthritis in DBA/1 mice as animal model of rheumatoid arthritis.

Particularly concerning methods to quantify inflammation alternative to the arthritis research gold standard, i.e. the semi quantitative histological assessment of disease severity, PET/CT imaging is offering new fields of application by *in vivo* measurement.

Inflammation of joints resulted in an increased tissue uptake of ^{18}F-FDG in fore and hind paws in stages of beginning or manifested disease, whereas no accumulation of ^{18}F-FDG was detectable before arthritis onset. Coincidence of ^{18}F-FDG PET signaling and clinical assessment of arthritis showed a statistically significant correlation, demonstrating capability of PET/CT as a quantitative *in vivo* imaging technique not only in tumor diagnosis and monitoring, but also in experimental arthritis research (manuscript in preparation).

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PET/CT zur Darstellung von Infektion und Entzündung: Reduction, Refinement und Replacement von Tierversuchen
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Selected publications
(HKI authors in bold)

Erfurth F, **Tretyakov A**, Nyuyki B, **Mrotzek G**, Schmidt WD, Fassler D, **Saluz HP** (2008) Two-laser, large field hyperspectral microarray scanner for the analysis of multicolour microarrays. *Analytical Chemistry* 80, 7706-7713.

Ibanez A, Schüler T, Möller R, Fritzsche W, **Saluz HP**, Svatos A (2008) DNA detection using a novel triple read-out optical/AFM/MALDI planar microwell plastic chip. *Analytical Chemistry* 80, 5892-5898.

Schmidt U, **Wollmann Y**, **Franke C**, Grosse F, **Saluz HP**, **Hanel F** (2008) Characterization of the interaction between the human DNA topoisomerase IIbeta-binding protein 1 (TopBP1) and the cell division cycle 45 (Cdc45) protein. *Biochem J* 409, 169-177.

Mihatsch K, **Nestler M**, **Saluz HP**, Henke A, **Munder T** (2009) Proapoptotic protein Siva binds to the muscle protein telethonin in cardiomyocytes during coxsackieviral infection. *Cardiovasc Res* 81, 108-115.

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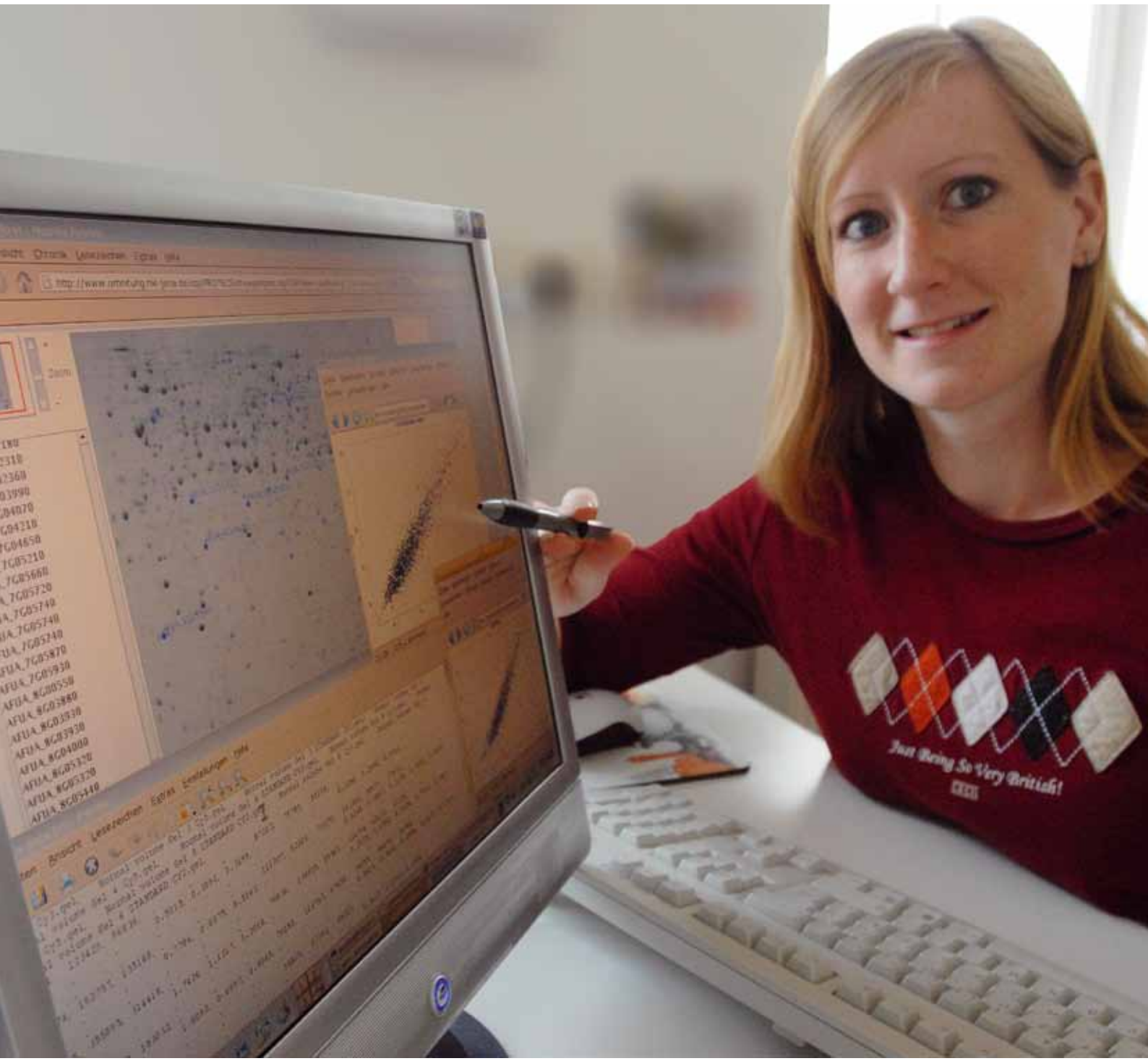

Research Group Systems Biology and Bioinformatics

Research Group Systems Biology and Bioinformatics

Bioinformatics at the HKI is devoted to progress in the emerging field of systems biology of fungal infections. To better understand and, finally, control an infection process, systems biology intends to consider not so much the individual behavior of a single gene or protein, but the collective behavior and interaction of the entirety of genes and proteins. Systems biology aims to infer and model relationships between molecular entities in gene regulatory network, in metabolic and signaling pathways as well as between cells on the population, organ or organism level. The goal of systems biology and bioinformatics is to discover structures and to understand the dynamics of biological processes by knowledge- and data-based modeling and purpose-driven model simulations.

The introduction of functional genomics technologies, in particular microarray technology, has allowed to monitor expression levels of thousands of genes and proteins during a dynamic biological process. Biological data is collected in many hundreds of different experiments where each consists of thousands of data. They are published in scientific journals and stored in several databases in different data formats and abstraction layers. The challenge for bioinformatics is to preprocess, analyze and model these data consistently. By the integration of the information from several experiments new scientific findings can be obtained with the help of methods from systems biology and comparative analysis.

INTRODUCTION | EINLEITUNG

Head:
PD Dr. Reinhard Guthke

Ein Schwerpunkt der Forschung am HKI ist die Systembiologie human-pathogener Pilze. Damit sollen Interaktionen zwischen dem Wirt, insbesondere dem Immunsystem, und Komponenten der pilzlichen Erreger als zwei kommunizierende Netzwerke beschrieben und in ihrer Dynamik analysiert werden. Die Netzwerke sind Ergebnis einer Daten- und Wissens-getriebenen mathematischen Modellierung. Das Wesen der Systembiologie ist nicht mehr nur die Untersuchung einzelner Moleküle, Reaktionen oder Zellen, sondern die Erforschung des Zusammenhangs möglichst aller am Infektionsprozess beteiligten Molekülen, Reaktionen und Zellen. Diese Zusammenhänge werden in Netzwerk-Modellen der Genregulation, der intra- und extrazellulären Kommunikation über Signalmoleküle und des Stoffwechsels abgebildet. Die Entwicklung neuer molekularbiologischer Messtechniken, wie die Mikroarray-Technik, ermöglicht es, die Expressionsintensitäten von Tausenden von Genen, Proteinen und Metaboliten während des biologischen Prozesses, insbesondere dem Infektionsprozess, in ihrer Dynamik zu beobachten. Durch Einsatz bioinformatischer Methoden können aus solchen Hochdurchsatzdaten die zugrundeliegenden genregulatorischen und Signal-Netzwerke rekonstruiert werden.

Die Analyse solcher Netzwerk-Modelle soll dann dazu beitragen, neue und bessere Therapie-Konzepte, neue therapeutische Targets und Biomarker für das Therapiemonitoring zu finden.

Bioinformatik am HKI leistet zu Entwicklung der Systembiologie einen wesentlichen Beitrag, indem sie durch Analyse der immer umfangreicher und komplexer werdenden experimentellen Daten neue wissenschaftliche

The research of the Systems Biology / Bioinformatics Group at the HKI is focused on management and analysis of genome-wide data, i.e. genomic, transcriptomic, proteomic and other high-throughput data, together with microbiological and/or clinical data. The data analysis is directed to the dynamic modeling of gene regulation and signaling associated with fungal infection processes and other selected biomedical as well as biotechnological processes. The generated computational models can be used to control or optimize experiments, biotechnical product formation and medical processes in diagnostics and therapy.

The data analysis pipeline at the HKI comprises storage and management of heterogeneous experimental data in a data warehouse, data pre-processing, discovery of the main features and patterns and – finally – generation of testable model hypotheses and model-based experimental design to verify the hypotheses. The hypotheses have been embedded in predictive mathematical and computational models that represent

(i) the available expert knowledge,
(ii) facts extracted from biological databases and
(iii) the hypotheses generated by experimental data analysis.

We generate quantitative dynamic and/or qualitative temporal models mainly from time series data by dynamic data mining techniques, in particular clustering and network inference algorithms. Some of these tech-

Hypothesen generiert und so die Ausrichtung der nachfolgenden experimentellen Arbeiten unterstützt.

Ein Schwerpunkt der Forschungen der Systembiologie/Bioinformatik–Gruppe am HKI ist die Analyse von Genexpressionsdaten, um daraus Netzwerkmodelle der Genregulation und Signaltransduktion zu gewinnen, die bei Infektionsprozessen und anderen biomedizinischen oder biotechnologischen Prozessen eine wesentliche Rolle spielen. Die so konstruierten Modelle können eingesetzt werden, um die therapeutischen oder biotechnologischen Prozesse zu steuern, um Experimente oder Prozesse der Naturstoff-Produktion zu optimieren oder auch zunächst für die Diagnose dieser Prozesse.

In der Forschungsgruppe werden Genom-, Transkriptom- und Proteomdaten sowie andere Hochdurchsatzdaten zusammen mit mikrobiologischen und klinischen Daten analysiert, um Netzwerkmodelle der Genregulation sowie der intrazellulären Kommunikation und Zell-Zell-Interaktionen mathematisch und auf dem Rechner zu modellieren. Der Arbeitsfluss der Datenanalyse umfasst die Datenspeicherung und das Datenmanagement von heterogenen experimentellen Daten in einem sogenannten „Datenwarenhaus“. Er schließt die Datenvorbehandlung, die Extraktion wichtiger Merkmale und Muster in den Daten ein und geht schließlich über die Vorhersage von neuen und experimentell überprüfbaren Hypothesen bis zum Modell-basierten Entwurf von weiteren Experimenten. Mit diesen können die Hypothesen geprüft werden und somit neues Wissen über den untersuchten Prozess gewonnen werden. Die Hypothesen sind dann eingebettet in mathematische Modelle und sind implementiert in eine Software-

niques have been developed – in collaboration with other groups – by our own work. The first steps were done to include also spatiotemporal data by image analysis. Our research focuses on inference (also referred to as reverse engineering) of gene regulatory network (GRN), i.e., the reconstruction of dynamic network models from observational data, in particular time-resolved transcriptome and proteome data (Hecker *et al.*, 2009a). The analysis of inferred molecular networks aims to contribute to rationally and efficiently develop biomarkers for improved and individualized therapeutic strategies.

The Research Group was and is involved in local and national systems biological research networks, in particular the Jena Centre for Bioinformatics (JCB), the Jena School for Microbial Communications (JSMC), the International Leibniz Research School for Microbial and Biomolecular Interactions (ILRS), the Jena Centre for Systems Biology of Ageing (JenAge), two German competence networks on Systems Biology (HepatoSys and FORSYS), the DFG Priority Program 1160 'Colonization and infection by human-pathogenic fungi' as well as the European network 'Nature inspired Smart Information Systems' (NiSIS).

INTRODUCTION | EINLEITUNG

Umgebung, wobei diese Modelle außer der zu testenden Modellhypothese auch das vorhandene Expertenwissen und das in Datenbanken gespeicherte Vorwissen repräsentieren.

Wir gewinnen quantitative oder qualitative dynamische Modelle hauptsächlich aus zeitlich aufgelösten Daten, sogenannten Zeitreihen, durch Anwendung spezieller bioinformatischer Algorithmen, insbesondere der Clusteranalyse und Netzwerkinferenz. Einige dieser Algorihmen wurden in der Forschungsgruppe selber – in Kooperation mit anderen Gruppen ausserhalb des Instituts - entwickelt. Die ersten Schritte wurden gegangen in Richtung raumzeitlich aufgelöster Modelle durch Anwendung von Algorithmen der Bildanalyse.

Die Forschungsgruppe ist integriert in verschiedene lokale und nationale Verbünde der systembiologischen Forschung, insbesondere dem Jenaer Centrum für Bioinformatik (JCB), der Jena School for Microbial Communications (JSMC), der International Leibniz Research School for Microbial and Biomolecular Interactions (ILRS), dem Jenaer Centrum für Systembiologie des Alterns (JenAge), zwei Deutschen Kompetenznetzwerken der Systembiologie (HepatoSys and FORSYS), dem Schwerpunktprogramm „Kolonisation und Infektion human-pathogener Pilze“ (SPP1160) der Deutschen Forschungsgemeinschaft (DFG) sowie dem Europäischen Netzwerk 'Nature inspired Smart Information Systems' (NiSIS).

1 The data warehouse OmniFung

Group Leader: Daniela Albrecht

The data warehouse is a data management system that aims at providing a platform for the storage, integration, retrieval and dissemination of the experimental data generated by different research groups. Primarily, OmniFung was developed in our group within and for the consortium of the priority program 1160 of the German Research Foundation (DFG) entitled 'Colonisation and Infection by Human-Pathogenic Fungi'. It includes also data from other (external) databases. OmniFung is also used by 'Fuminomics', a European community of 18 laboratories from 8 different countries active in research on 'Functional genomics in *Aspergillus fumigatus* and new strategies to fight against the first fungal pathogen in Europe'. In near future, OmniFung will be used and further developed by the competence network 'Septomics' established at the University of Jena.

Aim of the data warehouse development

OmniFung was developed to store and share transcriptome (microarray) and proteome (2Dgel electrophoresis (2D-GE) including mass spectrometry) data. Transcriptomic and proteomic data are connected via genomic data that are derived from NCBI RefSeq and UniProt and are additionally stored in the database. Each microarray or 2D-gel entry in the database includes an image and quantitative data of the spots of the image. In OmniFung, it is possible to view these images and to choose spots within them to see available information to this spot.

Our strategy during developing OmniFung was to be close to the needs of the user, store and analyze the data in standardized manner by using or defining Standard Operation Procedures, to reuse already established and successfully evaluated software, connect the database with other databases and (e.g. BMBF and EU funded) systems biology initiatives, such as FORSYS and EraSysBioPlus. Being close to the user means to incorporate the user into the definition of solutions. Researchers do not want to spend substantial amounts of their working time feeding databases. Thus, the data management solutions have to be easy to use. The data management system should support the scientist in the dissemination of the data within the community of project partners in a secure and controlled manner.

There is a public access via **www.omnifung.hki-jena.de**. Publicly available datasets are accessible without using a login and password. Presently, 35 registered users have a password-protected access to certain proprietary data subsets. Each researcher has full control over the access rights of his own projects.

Data structure

OmniFung stores transcriptome and proteome data together with extensive experimental annotations in standardized manner. The annotation parameters follow MIAME and MIAPE guidelines. To help users of the data warehouse in annotating their experiments, two Excel sheets were developed representing proteomic and transcriptomic parameters, respectively. These sheets include the different parameters of each hierarchical level of the data warehouse (i.e., project, cultivation, sample, array/gel type, array/gel, array/gel image) together with a short description. Most of the parameters are realized as selection lists. Such lists ensure that a controlled vocabulary is used to describe the experiments. The parameters can also serve as a quality measure of a project in OmniFung. A project is best understandable to others if all parameters are annotated. Therefore, on the project page in the database, the percentage of annotation is given showing the

user how careful the owner of the project was in annotating.

The import and export of data into a data warehouse must be user friendly, quick and reliable. The import of genomic data into OmniFung can only be done by the administrator of the data warehouse. This restriction ensures consistency of the entries. Currently, OmniFung contains data from four fungal species: *Aspergillus fumigatus*, *Candida albicans* and *Candida glabrata* as most important human-pathogenic fungi and *Aspergillus nidulans* as a model organism for filamentous fungi. Data from human and mice will follow in near future to study interactions of pathogens with hosts.

Importing of experimental data is possible for every registered user of the data warehouse. Microarray and gel images can be uploaded directly via the web interface. A number of image formats is possible including gif, jpeg, png, and tiff. Most image analysis programs create tiff-files because this format provides the most detail in images. The import of quantitative microarray data into the database is also possible via the web interface. Five formats are currently supported: GenePix Result (GPR), ArrayVision output, ScanArray Express output, and Affymetrix' CEL-files.

For 2D-GE data, there are two different possibilities for importing data. First, data from the Decodon software Delta2D can be uploaded via the web interface. Additionally, it is possible to use OmniFung as data pool within the software. This way, all changes made in spot matching, quantitation or labeling within Delta2D are immediately visible within OmniFung and vice versa. Second, XML workspace data from Image Master 2D Platinum and DeCyder can also be imported into the data warehouse.

Whichever format of data is inserted into the database, spot labels are evaluated for fitting to gene and protein names of OmniFung. If an appropriate gene or protein is found, then the spot data are connected to it and the imported microarray or gel can be found at the gene/protein information web page.

The export of experimental data from within a database is the foundation for re-analyzing data. Data can be downloaded in the original format or in MIAME/MIAPE compliant XML formats. Up- or downregulation of spots (i.e., transcripts or proteins of interest) on several microarrays or 2D gels can be visualized by numerical display as well as sparklines and heatmaps.

Data processing

The data warehouse does not only contain raw data but also provides web-based analysis that is part of a 'Standard Operation Procedure' for transcriptome and proteome analysis that represent the status quo of bioinformatics methods in the area of fungal research. Our strategy of reuse of software is put into practice by the fact that the data warehouse OmniFung is based on the Protecs system developed by Decodon (www.decodon.com/Solutions/Protecs), which itself is based on the object-relational database system Caché, and, for advanced data analysis, we primarily use public available Bioconductor and other R-packages. Nevertheless, some interfaces and tools had to be written by us.

Datasets in OmniFung can be browsed or a search function can be used. Browsing allows getting an overview of the different projects. The search functionality provides the possibility to look for certain kinds of data. In the genomics section, the name, synonym or cross-reference to another database of every gene and protein can be used for searching.

A user interested in a certain gene or protein can find all information to it within seconds, including the microarray or gel where the respective transcript or protein was detected on. Arrays/gels and array/gel images can be searched by their names. Within projects, cultivations and samples, all experimental parameters that were used to annotate database entries can be used to search for specific experimental settings.

Additional to the internal tools for analysis and visualization, the following tools are attached to the data warehouse: *DIGE analyzer*,

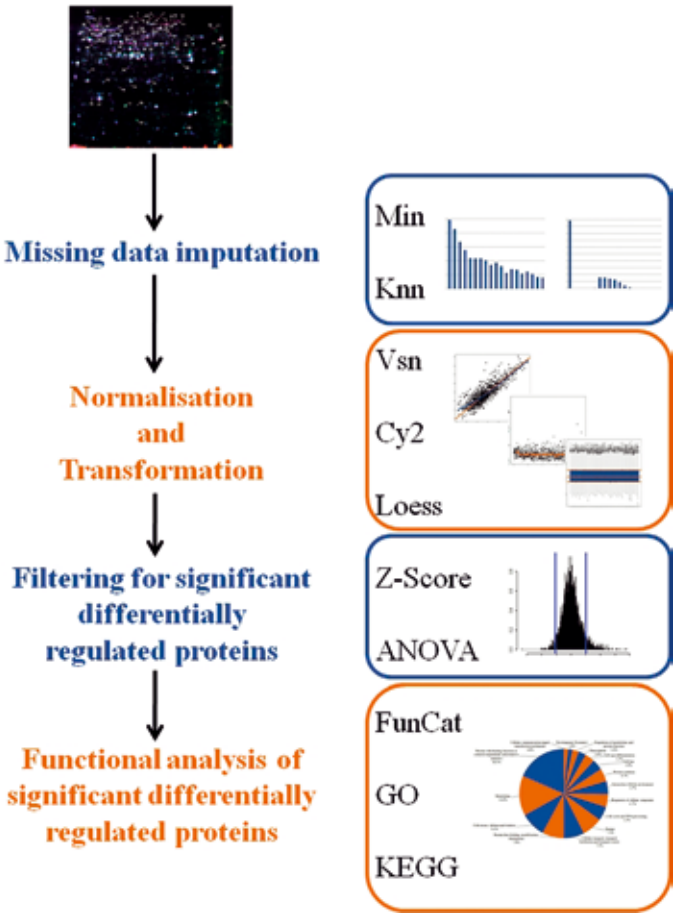

Figure 1
Workflow for transcriptome and proteome data analysis as implemented in the data warehouse OmniFung and applied for research in systems biology of human-pathogenic fungi.

Distance Scan, *FungiFun*, and *Survival Analysis*. *DIGE analyzer* (www.omnifung.hki-jena.de/Rpad/DIGE analyzer.Rpad) is a tool for preprocessing 2D-geldata, in particular for imputing missing values (Albrecht *et al.*, 2010a) and normalization of these data as well as for filtering differentially regulated proteins.

Distance Scan (www.omnifung.hki-jena.de/Rpad/Distance Scan/index.htm) is a tool for prediction of potential functional combinations of transcription factor binding sites (TFBSs). FungiFun (www.omnifung.hki-jena.de/Rpad/server/FungiFun/FungiFun.cgi) assigns functional annotations to fungal genes or proteins.

Systems biology of fungi

The HKI investigates the pathobiology of infections caused by human-pathogenic fungi. Pathogenicity of fungi is a multifactorial process. Therefore, genome sequence, transcriptome and proteome data are stored in the data warehouse OmniFung and analyzed for the elucidation of virulence determinants. Our data analysis workflow starts with pre-processing including imputing of missing values (Albrecht *et al.*, 2010a) and normalization and is followed by identification of differentially expressed genes/proteins as interesting candidates for further analysis, in particular for functional categorization and correlation studies (Figure 1). Sequence data and other prior knowledge extracted from databases are integrated to support the inference of gene regulatory networks associated with pathogenicity. This knowledge-assisted data analysis aims to establish mathematical models with predictive strength to assist further experimental work for testing novel hypothesis. Finally, the analysis of network models will be used to identify potential biomarkers and drug targets and, thus, assist the improvement of diagnosis and individualization of treatment of infectious diseases.

Hitherto, this integrative data analysis workflow up to dynamic modeling was applied by our group for the human-pathogenic fungi *Aspergillus fumigatus* and *Candida albicans*.

Aspergillus fumigatus

Aspergillus fumigatus has become the most important airborne fungal pathogen of humans causing pneumonia and invasive disseminated disease with high mortality in the immunocompromised hosts. *A. fumigatus* is a thermotolerant human-pathogenic mould. The thermotolerance of *A. fumigatus* is a trait which has been assigned to pathogenicity because it allows the fungus to grow in the human host even at higher temperatures. To elucidate this unique temperature resistance, the change of the fungal proteome during a temperature shift from 30 °C to 48 °C was analyzed by applying 2D-fluorescence difference gel electrophoresis (DIGE). The data were provided by HKI's Department Molecular and Applied Microbiology. Using our data analysis pipeline (Figure 1), the outcome of the image analysis was optimized by missing value imputation and normalization. Differentially regulated proteins were functionally classified and compared with already published transcriptome data of *A. fumgiatus* by applying correlation analysis methods. The study was completed by promoter studies of genes whose corresponding proteins were differentially regulated upon heat shock (Albrecht *et al.*, 2010b).

A number of 91 differentially regulated protein spots were identified by mass spectrometry representing 64 different proteins. They showed either a continuous up- or down-regulation or an oscillating behavior. Many proteins with a significant change in abundance represented proteins involved in protein folding (chaperones), oxidative stress response, signal transduction, transcription, translation, carbon- and nitrogen metabolism. A correlation of regulation between transcripts and the corresponding proteins was detected for half of the significant differentially regulated proteins provided that a time delay effect was introduced for the translation of transcripts.

The fungal adaptation to high temperatures revealed many similarities but also some obvious differences in the heat shock response compared to well-studied yeasts such as *Saccharomyces cerevisiae* and *S. pombe*. The heat shock response in *A. fumigatus* was transient and most changes in protein expression appeared within two hours. Afterwards, the level of protein expression sustained on a different, often higher level (e.g. heat shock proteins - HSPs) or dropped to the initial level (e.g. glycolytic enzymes). Besides the well known increased biosynthesis of HSPs upon heat stress, several other processes were differentially regulated: the oxidative stress response, signal transduction, transcription, translation, energy generation, carbohydrate and nitrogen metabolism and the cytoskeleton organisation.

The study was complemented by sequence analysis. Thus, novel putative targets of the heat shock regulator Hsf1 were predicted for mannitol biosynthesis, translation, cytoskeletal dynamics and cell division. Some of them seem to be *A. fumigatus* specific and mediate oxidative stress resistance in mitochondria, function as osmoregulator or ROI scavenger or which are required for nuclear migration. One could speculate that some of the putatively Hsf1-regulated target proteins confer also stress resistance during host infection.

Recently, first steps were done to extend the integrative data analysis and computational modeling by including and evaluating spatiotemporal data (movies) that monitor interactions of *A. fumigatus* components (e.g. conidia) with host immune cells. The Definiens Cognition Network Technology® is applied to screenings of different *A. fumigatus* mutants with respect to diverse phagocytosis behavior. Cell-tracking of human immune cells, e.g. macrophages and neutrophiles, in time-lapse microscopy images are studied as well. As the result, Definiens Cognition Network stores all objects, sub-objects and their semantic relationships in a clear hierarchy. In that way, the contextual information contained in the network enables the automated extraction of information analogue to the way a human brain

makes sense of the image. The network is described and built via a set of rules which in turn is programmed using a high level script (Cognition Network Language).

Candida albicans

Candida albicans is a harmless commensal yeast living in most warm blooded animals. However, the fungus can change its behavior to an aggressive pathogen within immunocompromised patients or in individuals with disrupted homoeostasis of the host flora. Commonly patients suffer from superficial or muscusoal infections, but the fungus is also able to enter the bloodstream and cause systemic infections with high mortality rates. The number of infections and mycoses associated mortality has been dramatically increasing within the last decades, mainly because of an growing number of susceptible individuals (AIDS, organ transplantation, major surgery, chemotherapy, aging society ...). Strikingly, *C. albicans* is able to adapt to a wide range of environments (pH, nutrient shift, temperature ...) and can virtually infect every human organ.

To elucidate molecular mechanisms of adaptation of *C. albicans* of different environmental conditions, we inferred gene regulatory networks from gene expression time series data from microarrays monitoring the response of *C. albicans* to human oral infection. Such data were obtained by Zakikhany *et al.* (2007) using reconstituted human oral epithelium (RHE) and were provided by HKI's Department Microbial Pathogenicity Mechanism to our group for the purpose of GRN network inference. Figure 2a shows the mean kinetics of the expression profiles of 1382 differentially expressed genes grouped by fuzzy c-means clustering into 6 clusters. The original data was measured at 5 time points (1, 3, 6, 12, 24 hours) after infection. The mean expression kinetics averaged over the genes belonging to the respective cluster were employed for network inference using our software tool NetGenerator with integration of prior knowledge about TFBSs (Figure 2b). Some of the predicted gene regulatory relationships are validated by literature, while others reveal yet unknown biological relevant interactions. For example, a number of target genes for the transcription factors Rim101p and Tup1p are newly predicted.

Aspergillus nidulans

Moreover, we applied methods of computational systems biology also to investigate the secondary metabolism of fungi that produce of antibiotics (Schroeckh *et al.*, 2009; industrial projects).

Aspergillus nidulans is a filamentous fungus which is able to form sexual spores through meiosis. It is therefore used to research the eukaryotic cell biology. *A. nidulans* has been completely sequenced and there has been great progress in elucidation of the molecular regulation of the biosynthesis of secondary metabolites.

We developed a novel microarray probe design for gene expression profiling studies with *A. nidulans*. The newly designed microarrays are currently applied to investigate the transcriptome under different environmental conditions. To improve the quality of the obtained data, published microarray data for *A. nidulans* are now integrated and methods which mine gene expression across different experiments are currently developed and employed. In particular, sophisticated methods for the integrated analysis of gene expression and metabolic flux analysis will be developed and applied to the secondary metabolite producer *A. nidulans*.

2 Sequence analysis, genome structure and genome mining in fungi

Group Leader: Ekaterina Shelest

In the field of sequence analysis applied to fungal genomes we work in four main directions:

1. Sequencing and functional annotation of the genomes of dermatophytic fungi *Arthroderma benhamiae* and *Trichophyton verrucosum*
2. Characterization of fungal transcription factors

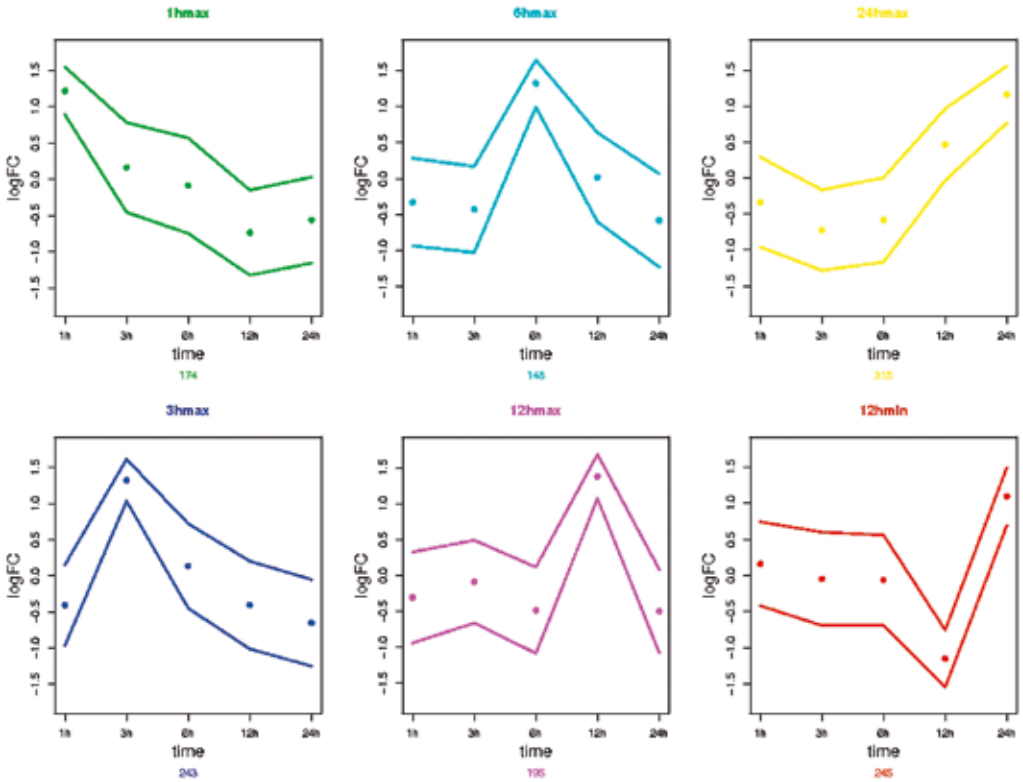

Figure 2a
The best cluster result groups the 1350 differentially expressed genes of *C. albicans* during oral infection into 6 clusters (dots: averaged values, lines standard deviation).

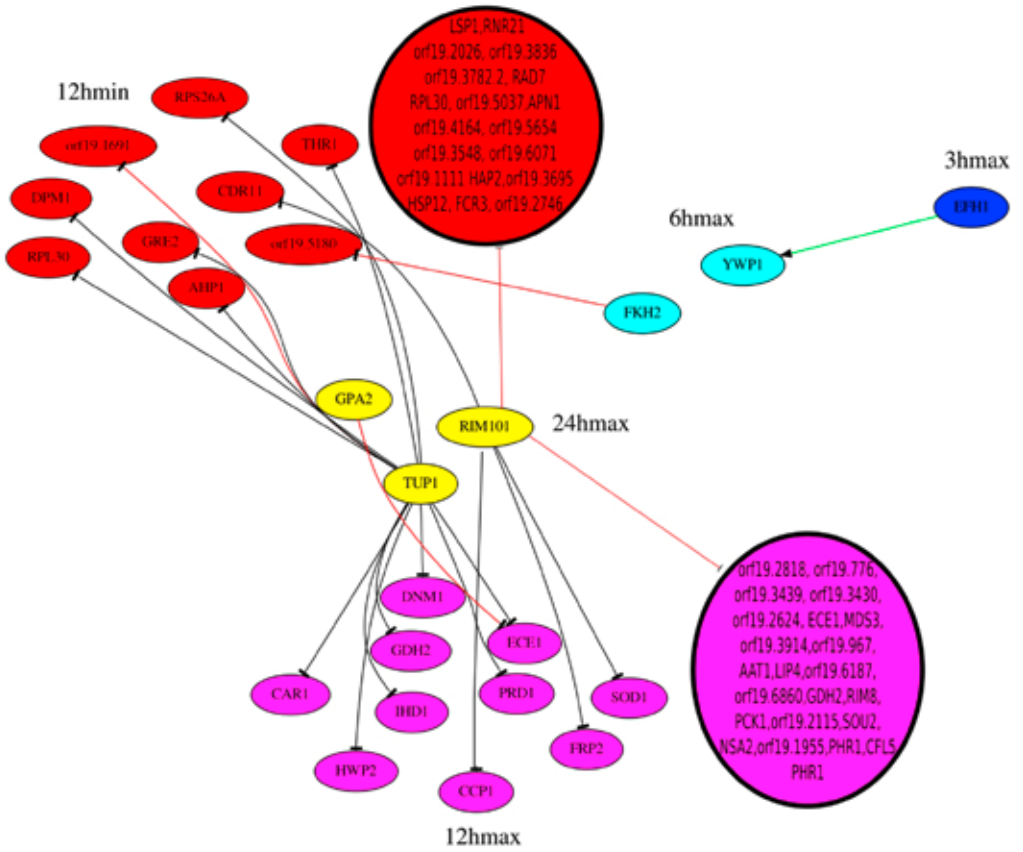

Figure 2b
Gene regulatory network in *C. albicans* at cluster basis (Figure 2a) inferred with the help of prior knowledge

3. Structural and functional characterization of the secondary metabolite gene clusters
4. Evolutionary aspects

Comparative and functional genomics of the dermatophytic fungi *Arthroderma benhamiae* and *Trichophyton verrucosum*

Dermatophytes are highly specialized pathogenic fungi and the most common cause of fungal skin infections. These fungi grow exclusively on keratinized tissues. Molecular tools are poorly developed and little is known about the molecular basis of pathogenicity of dermatophytes. The most important questions in this area concern the specificity of the host-pathogen interaction: What makes dermatophytes so efficient in infecting cutaneous tissue? Why do these fungi cause almost exclusively superficial infections, but very rarely deep-seated or systemic infections? Which factors are responsible for skin infections? Which common pathogenicity-associated traits may be shared by closely related dermatophytes? Why do closely related species exhibit differences in growth behaviour and different host preferences? To identify common traits necessary for colonisation of keratinized tissue and also differences responsible for host preferences, we sequenced the genomes of two dermatophyte representatives, *Arthroderma benhamiae* and *Trichophyton verrucosum* (this work has been done within the frame of the *Arthroderma benhamiae* genome project). In this first report on dermatophyte genomes, we found that 90% of the ~22.5 megabase genome sequences of *A. benhamiae* and *T. verrucosum* are unambiguously alignable and syntenic. This finding is remarkable given their dissimilarities in host specificity. Consistently, several proteins were only encoded by one of the genomes. Both fungi exhibit outstanding metabolic capacity for protein degradation since each genome contains about 230 predicted protease-encoding genes that include a newly-defined group of dermatophyte-specific proteases. The abundance of proteases in the genomes of *T. verrucosum* and *A. benhamiae* implicates a similar tissue adaptation. The expression of virulence attributes in *A. benhamiae* was validated by (i) secretome analysis of the fungus growing on keratin, and (ii) broad-scale transcriptome profiling during fungal–human keratinocyte interactions.

Characterization of fungal transcription factors

Transcription factors (TFs) orchestrate gene expression control of a cell and, in many respects, their repertoire determines the life and functionality of the cell. For a better understanding of their regulatory mechanisms, it is essential to know the entire repertoire of TFs of a species. The increasing number of sequenced genomes together with the development of computational methods allow us not only to predict whole sets of TFs but also to analyse and compare them. Such an analysis is required in particular for fungal species, as our knowledge of the potential set of TFs in fungi is very limited. In fact, at present we do not know which TFs can in general be found in fungi, and which of them are strictly fungal specific. Other interesting questions regard the evolutionary relationships of fungal TFs with other kingdoms and the functions of fungal-specific TFs. We undertook a screening of fully sequenced fungal genomes the known DNA-binding domains. The analysis of the occurrences of DNA-binding domains in 62 fungal genomes reveals a set of 37 potential 'fungal' TF families. Six families are fungal-specific, i.e. they do not appear in other kingdoms. Interestingly, the fungal-specific TFs are not restricted to strictly fungal-specific functions. Consideration of fungal TF distributions in different kingdoms provides a platform to discuss the evolution of domains and TFs.

Although the research in the field of transcription regulation in fungi is very active, there is no database of fungal transcription factors so far. Therefore, we developed a new database, FungiTF, where we collect the experimentally verified TFs and their binding sites reported in literature. The database is manually curated and currently contains 114 genes, 118 binding sites and 88 transcription factors. It will be publicly available this year.

Structural and functional characterization of the secondary metabolite gene clusters; evolutionary aspects.

In this field, the work is done in several directions:

- Prediction of PKS and NRPS genes in known and newly sequenced genomes; prediction of their functionality; prediction of gene clusters;

- Transcription factors involved in the regulation of the secondary metabolite gene clusters; prediction of binding sites, over-represented motifs in the promoters of the gene clusters;

- Phylogenetic relationships secondary metabolite biosynthesis genes (Sproete *et al.*, 2008, Schroeckh *et al.*, 2009).

Gene regulatory network inference

Systems biology aims to model relationships between molecular entities, such as genes, proteins (e.g. TFs) and metabolites. There is a limitation in inferring the regulatory interactions between genes using expression data only, and hence it makes sense to integrate diverse types of data, e.g. genome sequence data and gene functional annotations, as well as prior biological knowledge, e.g. from scientific literature, to obtain more accurate models of gene regulation (Hecker *et al.*, 2009a). We applied different approaches to integrate both, experimental data and prior knowledge into the modeling of gene regulatory networks (GRNs) in human-pathogenic fungi (see above) as well as in human and murine cells and the model microorganisms *S. cerevisiae* and *Escherichia coli*.

Mainly we applied differential and difference equation based modeling techniques for small and medium size modeling. For genome-wide modeling of gene regulation, e.g. in *E. coli*, we applied an information theory based approach.

The international initiative called 'Dialogue on Reverse Engineering Assessment Methods' (DREAM) is fostering a concerted international effort by computational and experimental biologists to understand the limitations and strengths of techniques for inferring networks from high-throughput data. We participated in this international initiative applying a novel multi-objective network inference method for the DREAM challenge that provides a qPCR data set from a perturbed synthetic five gene network in *S. cerevisiae*.

We developed and published the novel algorithm TILAR (Transcription factor binding site-Integrating Least Angle Regression) for deriving GRNs from gene expression data by incorporating known or predicted transcription factor binding sites (TFBS) and, if available, literature mining information. Our linear, additive modeling approach distinguishes genes and TFs in the network, and identifies the connections between them based on the fast Least Angle Regression algorithm and specific constraints on the network structure. The advantage of this integrative inference strategy is that only a reduced number of model parameters are sufficient to describe a complex network, which is still easy to interpret in terms of true molecular interactions.

GRN inference in the human immune system

We applied TILAR to characterize the transcriptional program induced by two different autoimmune disease therapies. In the first study, we analyzed Affymetrix GeneChips® HUman Genome U133A data of 19 patients suffering from rheumatoid arthritis (RA) to uncover molecular mechanisms underlying the response to the anti-TNF-alpha therapy by Etanercept (collaboration with the Institute of Immunology, University Rostock, and the Clinic of Rheumatology, Magdeburg/ Gommern/ Vogelsang) (Hecker *et al.*, 2009b). The inferred GRN simulates the behavior of differentially expressed genes within the first week of therapy. The reconstructed GRN includes 95 nodes, i.e. 83 differentially expressed genes and 12 TFs, as well as 22 gene-TF and 84 TF-gene interactions (Figure 3).

In a similar study, we investigated the transcriptional changes within the first month of IFN beta-1a (Avonex) treatment of 24 patients

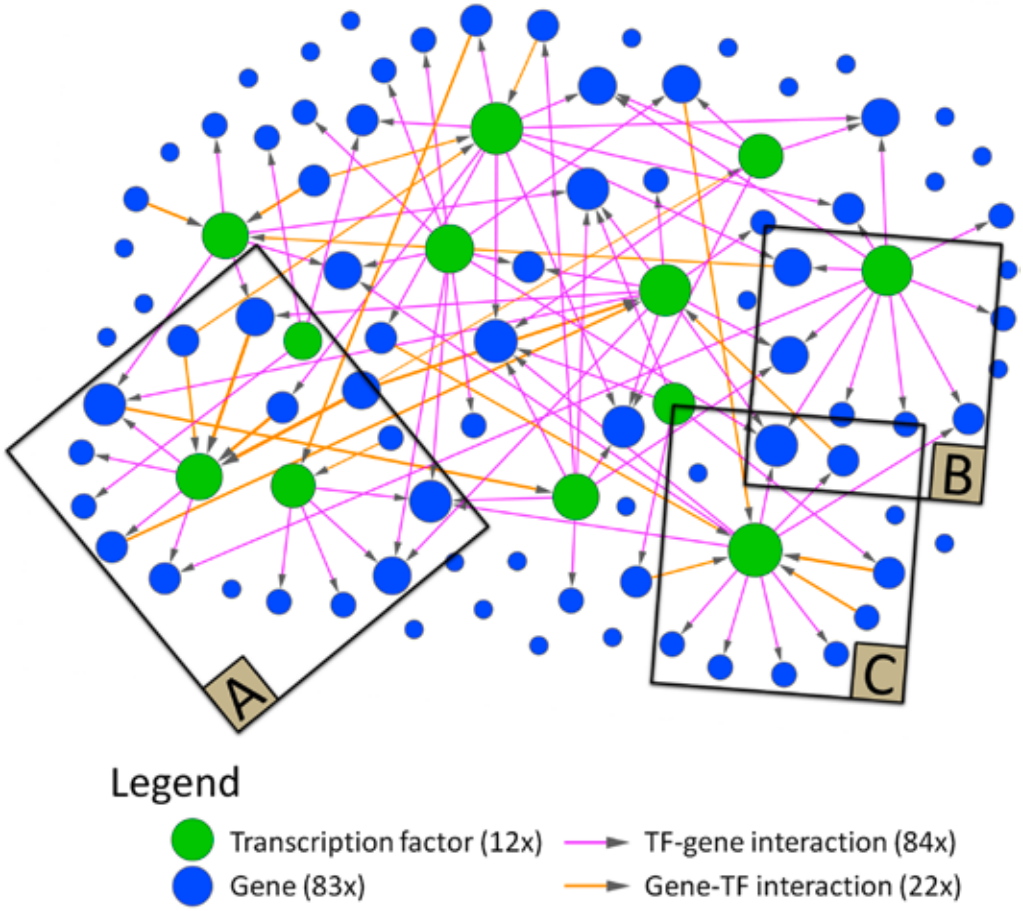

Figure 3
Gene regulatory network inferred by TILAR from gene expression data monitoring the response of peripheral mononuclear blood cells of RA patients towards anti-TNF-alpha therapy (Hecker *et al.*, 2009). The blue nodes represent the 83 genes differentially expressed genes within the first week of therapy. The 12 green nodes represent the predicted regulators, i.e. TFs which TF-binding sites are over-represented in the set of the differentially expressed genes. The size of the nodes corresponds to their degree of connectivity.

diagnosed with multiple sclerosis, and used TILAR to provide a molecular understanding of the therapeutic effects (collaboration with the Department of Neurology, University Rostock).

Moreover, we systematically evaluated the performance of the novel integrative GRN inference algorithm TILAR by comparison with the results obtained by application of five other inference methods that use gene expression data alone (CLR, ARACNE, GeneNet, Lasso, qp-graph). TILAR outperformed all other algorithms tested on both data sets. The R codes for TILAR is public available via the HKI-homepage (www.sysbio.hki-jena.de >> software).

In another study we inferred networks of molecular interactions of disease-related proteins (cytokines) that describes the transcriptional response of synovial fibroblasts from RA and osteoarthritis patients to stimulation by TNF-alpha and TGF-beta. Data from 120 Affymetrix GeneChips® Human Genome U133Plus2.0 arrays were analyzed to identify differentially expressed genes (collaboration with the Experimental Rheumatology Unit, University Hospital Jena). The preprocessed expression data of subsets of differentially expressed genes belonging to immune system-related pathways were employed for construction and simulation of ordinary differential equation (ODE) systems using the network inference tool NetGenerator (developed by Bio-Control Jena in collaboration with our group). Differential expression over the time course of certain matrixmetalloproteases (MMP1, MMP3, MMP10) was exclusively found in patients suffering from severe RA. A subset of the data was also used to establish and analyze a Boolean network model by formal concept analysis for the gene regulation representing the formation and destruction extracellular matrix in TNF-alpha and TGF-beta stimulated synovial fibroblasts of RA patients (Wollbold *et al.*, 2009).

References

Albrecht D, Kniemeyer O, Brakhage AA, Guthke R (2010a) *Proteomics* 10, 1–10.

Albrecht D, Guthke R, Brakhage AA, Kniemeyer O (2001b) *BMC Genomics* 11, 32.

Hecker M, Lambeck S, Toepfer S, van Someren E, Guthke R (2009a) *BioSystems* 96, 86–103.

Hecker M, Goertsches RH, Engelmann R, Thiesen HJ, Guthke R (2009b) *BMC Bioinformatics* 10, 262.

Schroeckh V, Scherlach K, Nützmann H-W, Shelest E, Schmidt-Heck W, Schuemann J, Martin K, Hertweck C, Brakhage AA (2009) *PNAS USA* 106, 14558–145563.

Shelest E (2008) *FEMS Microbiology Letters* 286, 145–151.

Spröte P, Hynes MJ, Hortschansky P, Shelest E, Scharf DH, Wolke SM, Brakhage AA (2008) *Mol Microbiol* 70, 445–461.

Wollbold J, Huber R, Pohlers D, Koczan D, Guthke R, Kinne RW, Gausmann U (2009) *BMC Systems Biology* 3, 77.

Zakikhany K, Naglik JR, Schmidt-Westhausen A, Holland G, Schaller M, Hube B (2007) *Cellular Microbiology* 9, 2938–2954.

GRN inference in stimulated hepatocytes

We inferred GRNs in murine hepatocytes from gene expression data monitoring the response to cultural media exchange (in collaboration with the Institute for Biochemistry, University Leipzig) simulating changes in the blood composition of the liver. The expression of genes at five different time points was recorded using Affymetrix GeneChip® MOE4302. The preprocessed expression data of selected differentially expressed genes belonging to pathways of the glycolysis/gluconeogenesis, nitrogen metabolism, glycerolipid metabolism, glutamate metabolism, pyruvate metabolism, pentose phosphate pathway, citrate cycle and synthesis and degradation of ketone bodies were employed to infer a network model based on a differential equation system using our NetGenerator algorithm under different network search strategies, such as network inference without prior knowledge, pre-setting of robust stimulus-gene interactions and the integration of biological knowledge. Methods of statistical model analysis such as parameter sensitivity and identifiability analysis were applied to find an adequate model.

In another study, transcriptome data monitoring partial hepatectomy induced murine liver regeneration were used for network model inference to better understand processes of liver regeneration. Data for two biological replicates covering nine timepoints were preprocessed and differentially expressed genes were determined. To the purpose of dimension reduction of data, TF-target gene interactions were derived from cisRED database and were fitted by a least square error based optimization procedure to the gene expression data. The findings concern regulatory influences of TFs and potentially new target genes within this model, in particular up-regulated Jun, Stat3 and Foxo1 genes, that code for TFs and are essential for the model of liver regeneration as shown by simulation and validated by another data set.

GRN inference in Escherchia coli

Gene expression profiles from the Many Microbes Microarray Database for *E. coli*, were employed for GRN inference using popular algorithms based on mutual and directed information (ARACNE, CLR, MRNET, DTI). The inferred networks have been evaluated by using known gene interactions from RegulonDB and by model-based directed experimental work. In collaboration with the University of Jena / Department of Bioinformatics, we identified 75 TF – target gene interactions of which 65 were known according to RegulonDB and further five have already been reported in the literature. One of the five remaining and predicted hitherto unknown influences, the regulation of lipoate synthase by the pyruvate-sensing pyruvate dehydrogenate repressor (PdhR), was experimentally checked and confirmed by the Department of Genetics of the University of Osnabrück.

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Teilprojekt: Systems biology of human pathogenic fungi: Modeling transcriptional networks of virulence
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Teilprojekt: Holistic approach to genomics of human-pathogenic fungi: Data warehouse for integration of data on transcriptome, proteome and metabolome of *Candida albicans* and *Aspergillus fumigatus*
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Selected publications
(HKI authors in bold)

Shelest E (2008) Transcription factors in fungi – Minireview. *FEMS Microbiology Letters* 286, 145-151.

Hecker M, Goertsches RH, Engelmann R, Thiesen HJ, **Guthke R** (2009) Integrative modeling of transcriptional regulation in response to antirheumatic therapy. *BMC Bioinformatics* 10, 262.

Hecker M, **Lambeck S**, Toepfer S, van Someren E, **Guthke R** (2009): Gene regulatory network inference - data integration in dynamic models - A review. *BioSystems* 96, 86-103.

Schroeckh V, **Scherlach K**, **Nützmann H-W**, **Shelest E**, **Schmidt-Heck W**, **Schuemann J**, **Martin K**, **Hertweck C**, **Brakhage AA** (2009) Intimate bacterial-fungal interaction triggers biosynthesis of archetypal polyketides in *Aspergillus nidulans*. *Proc Natl Acad Sciences USA* 106, 14558-14563.

Vödisch M, **Albrecht D**, **Leßing F**, **Schmidt AD**, **Winkler R**, **Guthke R**, **Brakhage AA**, **Kniemeyer O** (2009) Two-dimensional proteome reference maps for the human-pathogenic filamentous fungus *Aspergillus fumigatus*. *Proteomics* 9, 1407-1415.

Wollbold J, Huber R, Pohlers D, Koczan D, **Guthke R**, Kinne RW, Gausmann U (2009) Adapted Boolean network models for extracellular matrix formation. *BMC Systems Biology* 3, 77.

Albrecht D, **Guthke R**, **Brakhage AA**, **Kniemeyer O** (2010) Integrative analysis of the heat shock response in *Aspergillus fumigatus*. *BMC Genomics* 11, 32.

Albrecht D, **Kniemeyer O**, **Brakhage AA**, **Guthke R** (2010) Missing values in gel-based proteomics. *Proteomics* 10, 1202-1211.

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Research Group Pharmaceutical Biology

The department Pharmaceutical Biology is affiliated with the Pharmaceutical Institute of the Friedrich-Schiller-Universität and, at the same time, associated with the Leibniz Institute for Natural Product Research and Infection Biology - Hans-Knöll-Institute (HKI). The department thus represents another excellent example for the HKI's close interaction with the university. Head of the department is Associate Professor Dr. Dirk Hoffmeister who relocated to Jena in February of 2009. As a group focusing on pharmaceutical research our objective is to isolate and analyze microbial small molecule natural products as potential new pharmaceuticals or as chemical signals in microbial communication processes. Along with this endeavor, we elucidate the biochemical and genetic basis of natural product biosyntheses. A particular emphasis is placed on basidiomycete secondary metabolism, however, our projects also include bacterial small molecules.

As a university-affiliated group, the associated department Pharmaceutical Biology actively engages in teaching within the curriculum of the Pharmaceutical Sciences. Students can elect to fulfill the requirements for diploma theses or laboratory internships in the department and gain hands-on experience in natural product research early on.

INTRODUCTION | EINLEITUNG

Head:
Prof. Dr. Dirk Hoffmeister

Die Abteilung Pharmazeutische Biologie ist Teil des Institutes für Pharmazie der Friedrich-Schiller-Universität Jena, jedoch gleichzeitig dem Leibniz-Institut für Naturstoff-Forschung und Infektionsbiologie – Hans-Knöll-Institut assoziiert. Sie stellt damit ein weiteres Beispiel für die enge Verzahnung des HKI mit der universitären Forschung dar. Die Abteilung wird von Professor Dr. Dirk Hoffmeister geleitet und hat im Februar 2009 die Arbeit aufgenommen. Als eine pharmazeutisch ausgerichtete Gruppe betreibt sie Forschung über Naturstoffe mit dem Ziel der Isolation und Analyse von kleinen mikrobiellen Sekundärmetaboliten als potentielle neue Wirkstoffe oder Informationsträger für mikrobielle Kommunikation. Ein Schwerpunkt ist die Erforschung der genetischen und biochemischen Grundlagen, auf denen die jeweiligen zellulären Biosynthesewege dieser Sekundärmetabolite aufbauen. Hauptsächlich werden Ständerpilze (Basidiomyceten) untersucht, jedoch ist auch der bakterielle Stoffwechsel durch ein Projekt in der Gruppe repräsentiert.

Als universitäre Gruppe ist die Abteilung Pharmazeutische Biologie auch aktiv in Lehraufgaben zur Ausbildung von Apothekern eingebunden. Durch die Vergabe von Praktikums- und Diplomarbeiten sowie Plätzen für Wahlpflichtpraktika gemäss der Approbationsordnung für Apotheker kann der pharmazeutische Nachwuchs auf diese Weise frühzeitig an akademische Naturstoff-Forschung herangeführt werden.

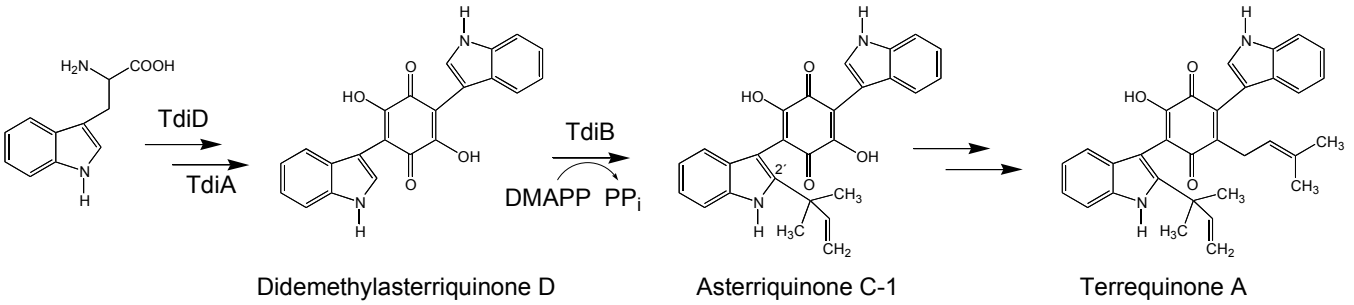


Figure 1
The biosynthesis of asterriquinones in *Aspergillus nidulans*.

1 Quinone pigments in fungi

Progress in the area of quinone pigments from filamentous fungi was made with two organisms: *Aspergillus nidulans* is the producer of terrequinone A (Figure 1), a member of the bisindolylquinone family of compounds. Their potential use for pharmaceutical purposes stems from antiretroviral activities and insulin receptor activation in mice after oral administration.

Earlier, the enzymes TdiA and TdiD were recognized as gateway enzymes which govern bisindolylquinone-backbone formation. TdiA represents a tri-domain enzyme reminiscent to non-ribosomal peptide synthetases yet lacking a condensation domain. TdiD ist a pyridoxal 5-phosphate (PLP)-dependent aminotransferase. These two enzymes convert L-tryptophan into didemethylasterriquinone D (Figure 1), the universal intermediate of all bisindolylquinones. Prenylation is a frequent post-assembly modification and contributes to molecular diversity and biological acitvity. The enzyme TdiB was biochemically characterized as a cation-independent prenyltransferase, which catalyzes asterriquinone C-1 formation by reverse prenylation of carbon atom 2' of didemethylasterriquinone D.

The second fungus pertinent to this project is *Tapinella panuoides*, a homobasidiomycete belonging to the Boletales and a known producer of atromentin (Figure 2). Atromentin represents the key intermediate for the entire terphenylquinone and pulvinic acid family of secondary products. These are common homobasidiomycete pigments, whose structural diversity is generated by numerous modifications, such as hydroxylation and subsequent heterocyclization, oxidative ring cleavage and rearrangement, hydroxylations, or esterification. Therefore, atromentin serves as a widely valid model to investigate the genetic and bio-

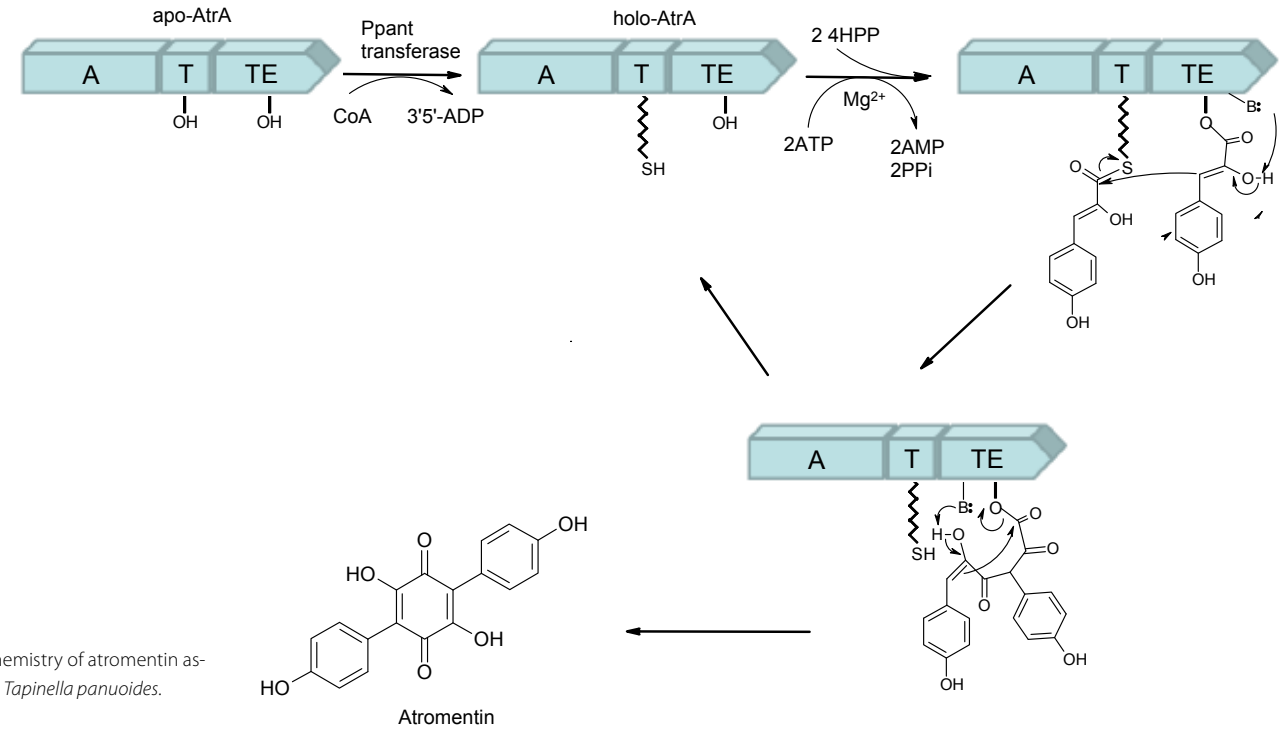


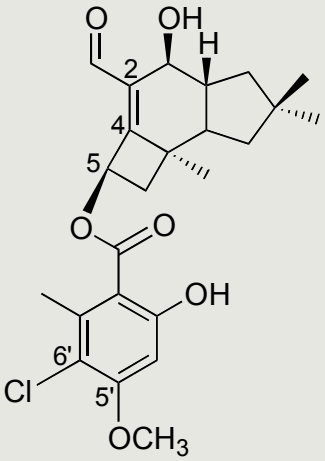
Figure 2
The biochemistry of atromentin assembly in *Tapinella panuoides*.

chemical basis of natural products in basidiomycetes.

Guided by the concept of *tdiA/tdiD*-encoded bisindolylquinone biosynthesis enzymes, orthologous genes for the atromentin synthetase AtrA and the L-tyrosine:2-oxoglutarate aminotransferase AtrD were detected in a genomic *Tapinella* cosmid library, and the enzymes biochemically characterized. By reconstitution of the pathway *in vitro* it was shown that AtrA and AtrD catalyze consecutive steps to turn over L-tyrosine into atromentin (Figure 2). Therefore, the synthesis of quinones from the side chains of aromatic 2-oxo acids was demonstrated as a conserved biosynthetic strategy in both asco- and basidiomycetes.

2 Melleolide natural products from *Armillaria* species

Many species within the homobasidiomycete genus *Armillaria* ("honey mushroom", Figure 3) are known as notorious butt and root rot agents and globally present as pathogens both in managed and unmanaged hardwood and conifer forests. The host range of *Armillaria* is extraordinarily broad and also includes fruit and ornamental trees. Although being an aggressive tree pathogen, *Armillaria* species also serve a positive role by depolymerizing biomass and, therefore, maintaining the carbon flux in forest habitats. Various studies established *Armillaria* species as producers of the melleolides (e.g., arnamial, Figure 4). These compounds represent biosynthetic chimera, composed of a sesquiterpenoid protoilludane whose alcohol functionality at position 5 engages in ester bond formation with orsellinic acid or a derivative thereof. Orsellinic acid is a common microbial metabolite, and protoilludanes are frequently found in basidiomycetes. However, the esterification of these two building blocks into a more complex metabolite is a unique biosynthetic capacity of *Armillaria*



Arnamial

Figure 3
Armillaria mellea carpophores

Figure 4
Chemical structure of arnamial

whose secondary metabolism is now investigated at the HKI. The pharmaceutical aspect of this project includes the inhibitory activities against human cell lines, which have not been described previously. A new member of the melleolides, arnamial, was isolated along with a number of known compounds of this family. By comparing the inhibitory effects, a preliminary structure-activity-relationship emerged. Our data points to the $\Delta^{2,4}$ double bond as being critical for cytotoxicity, while 6'-chlorination and 5-O-methyl ether formation contributed less. We also found that the mechanism of action of arnamial involves the apoptotic pathway.

Some filamentous fungi with which *Armillaria* is likely to interact in its natural habitat are transiently or permanently inhibited by the melleolides, while other fungi are completely resistant against them. This is suggestive of a role in chemical ecology and, more specifically, that *Armillaria* might have evolved these unique compounds as a means for selective negative interspecies communication. To study this aspect and to identify the molecular mechanisms of how the melleolides exert their inhibitory activity, a project within the Jena School of Microbial Communication (JSMC) has been established.

3 Natural products from an unidentified basidiomycete

An unusual homobasidiomycete was isolated from wood and has so far defied any taxonomic identification, both by morphological and genetic approaches. Interestingly, this fungus was only found once, attempts to retrieve the organism both at the initial site of collection and in related habitats failed. This basidiomycete exerts a strong negative intermicrobial communication: if colonized by this fungus wood is far less susceptible to growth of blue-stain fungi and other microbes. Therefore, the

Figure 5
Tomato plants killed by *Ralstonia solanacearum*

impact upon timber quality is reduced and, eventually, the market value maintained. Antimicrobial assays with crude extracts of the culture broth confirm inhibition, and significant antifungal activity was observed in the assay even after several weeks.

Together with partners in the Department of Plant Pathology at the University of Minnesota-Saint Paul, we have begun investigating the secondary metabolome of this unique fungus, with a particular emphasis on antifungal compounds and a potential application as biocontrol agent or a pharmaceutical use in mind. Supported by the HKI Bio Pilot Plant and embedded into the International Leibniz Research School for Microbial and Biomolecular Interactions (ILRS Jena) we currently i) establish large-scale fermentation conditions, ii) identify the mode of action of the antifungal principle and iii) isolate other secondary metabolites to chemotaxonomically complement genus/species identification efforts.

4 Secondary metabolism in *Ralstonia solanacearum*

Ralstonia solanacearum is a Gram-negative bacterium and notorious plant pathogen as it causes a lethal wilt disease (Figure 5). The host range of this organism is remarkably broad and includes plants used for staple food production, such as banana and potato, and species of significant commercial value, including tomato, eggplant, and tobacco. Given its relevance as pathogen, *R. solanacearum* warrants more profound investigations into its secondary metabolism which may be implicated in virulence and contribute to bacterial wilt disease. Previous studies identified the global virulence regulatory proteins VsrAD and PhcA as central in the regulation of diverse functions needed by *R. solanacearum* during growth inside host plants. VsrAD is a two-component regulatory system that broadly governs expression of many stress and virulence factors. PhcA is a LysR-type global virulence regulator that con-

trols exopolysaccharide production, bacterial motility, and several other virulence factors in response to a quorum-sensing mechanism. In collaboration with partners at the University of Wisconsin-Madison, VsrAD and PhcA deletion mutants were generated. In these engineered strains the secondary metabolism was reduced or disabled. The results led to identification of several new natural products whose chemical structures are elucidated at the HKI in cooperation with the Junior Research Group Secondary Metabolism of Predatory Bacteria. Based on transcriptomic data we currently focus on the identification of biosynthetic genes, and on the biochemical characterization of the corresponding enzymes.

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Selected publications
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Schneider P, Bouhired S, **Hoffmeister D** (2008) Characterization of the atromentin biosynthesis genes and enzymes in the homobasidiomycete *Tapinella panuoides*. *Fungal Genet Biol* 45, 1487-1496.

Schneider P, Weber M, **Hoffmeister D** (2008) The *Aspergillus nidulans* enzyme TdiB catalyzes prenyltransfer to the precursor of bioactive asterriquinones. *Fungal Genet Bio*l 45, 302-309.

Misiek M, **Hoffmeister D** (2008) mRNA processing sites involved in intron splicing of *Armillaria* natural product genes. *Mycol Res* 112, 216-224.

Misiek M, Williams J, Schmich K, Hüttel W, Merfort I, Salomon C, Aldrich CA, **Hoffmeister D** (2009) Structure and cytotoxicity of arnamial and related fungal sesquiterpene aryl esters. *J Nat Prod* 72, 1888-1891.

Schneider P, Jacobs J, Neres J, Aldrich CA, Allen CZ, **Nett M**, **Hoffmeister D** (2009) The global virulence regulators VsrAD and PhcA control secondary metabolism in the plant pathogen *Ralstonia solanacearum*. *ChemBioChem* 10, 2730-2732.

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Research Group Fungal Septomics (ZIK Septomics)

Research Group Fungal Septomics

Sepsis is a life-threatening systemic infection with a high mortality which affects people of all ages. Sepsis severely compromises the progress of medicine in many fields and consumes health care resources. The mortality of severe sepsis is over 50 % and has not changed much over the last decades. In order to accelerate advances in prevention, rapid diagnosis and treatment of patients with sepsis, new diagnostic and therapeutic approaches must be developed. This, however, requires integrated and interdisciplinary medical research on the molecular basis of sepsis and biological pathways that offer a strong promise for the development of therapeutic interventions. The interdisciplinary collaboration of functional genomics in patient and pathogens together with modern molecular and cellular biology within the ZIK Septomics forms the basis for such advances. The funding program “centers for innovation competence” (Zentren für Innovationskompetenz, ZIK) aims to support the expansion of scientifically excellent research clusters in East-German universities and research institutes to generate internationally visible centers (www.unternehmen-region.de). Within the ZIK Septomics the Jena expertise in sepsis research will be strengthened and expanded. For that purpose, the ZIK Septomics establishes a new structural basis for sepsis research. Until now, sepsis research has mainly been divided into different disciplines. To develop a holistic understanding of sepsis pathophysiology, Septomics brings together,

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Head:
Prof. Dr. Oliver Kurzai

Sepsis ist eine lebensbedrohliche Infektion, die den gesamten Organismus erfasst. Sie ist mit hoher Sterblichkeit verbunden, kommt immer häufiger vor und betrifft Menschen in jedem Lebensalter. Sepsis gefährdet den medizinischen Fortschritt in vielen Bereichen der Hochleistungsmedizin und verbraucht Ressourcen im Gesundheitswesen. Die Sterblichkeit der schweren Sepsis beträgt über 50 % und hat sich in den letzten Jahrzehnten nicht entscheidend reduziert. Um Behandlungsfortschritte zu erzielen, müssen neuartige Diagnostika und Therapeutika entwickelt werden. Voraussetzung für diese Innovation ist ein neues molekulares Verständnis der Sepsis. Die Grundlage für einen solchen innovativen pathophysiologischen Ansatz bietet die im ZIK Septomics erstmals erreichte interdisziplinäre Vereinigung der funktionellen Genomik von Patient und Erreger mit modernsten molekular- und zellbiologischen Techniken. Das Programm „Zentren für Innovationskompetenz (ZIK)“ dient der Vertiefung herausragender Forschungsansätze an ostdeutschen Hochschulen und Forschungseinrichtungen und der Schaffung international sichtbarer Zentren (vgl. www.unternehmen-region.de). Im ZIK Septomics soll die am Standort Jena vorhandene Expertise im Bereich Sepsisforschung verstärkt und ausgebaut werden. Das ZIK Septomics etabliert zu diesem Zweck eine neuartige Struktur der Sepsisforschung. Bisher wurde Sepsisforschung zu Teilaspekten und in getrennten Disziplinen betrieben. Um ein holistisches Verständnis der Sepsis zu entwickeln, bringt Septomics die Disziplinen Molekularbiologie, Mikrobiologie, Infektiologie, Genomanalyse, Bioinformatik, Labormedizin und Intensivmedizin zusammen, entwickelt eine gemeinsame umfassende Forschungsstrategie und stellt für die Forschergruppen des Zentrums ein neues Forschungsgebäude zur Verfügung. Hier werden die bisher weitgehend getrennten Bereiche Erreger- und Wirtsantwortsforschung komplementär bearbeitet und

molecular biology, microbiology, infection biology, genomics, bioinformatics, laboratory medicine and intensive care specialists in a universal research strategy. Within a new laboratory building, host and pathogen biology will be addressed in complementary approaches and linked to bioinformatics and clinical practice. Other singular possibilities at the ZIK Septomics include the availability of Biocollections including blood and serum specimens from patients as well as a direct link to basic science. Septomics is a faculty-spanning center of the University of Jena and is scientifically interconnected with the Department of Anaesthesiology and Intensive Care Medicine (KAI), the Institute for Medical Microbiology and the Leibniz Institute for Natural Product Research and Infection Biology (Hans-Knöll-Institute). Contrary to the classical view of sepsis as an overactivation of immune reactions, new pathophysiological concepts depict sepsis as a result of a complex immunological dysregulation including both hyperinflammatory and immunosuppressive stimuli. It is a well recognized fact that the spectrum of pathogens responsible for sepsis has changed dramatically over the last decades with Gram-positive bacteria and fungi (*Candida spp.*) becoming increasingly more important. In the US, *Candida* species have become the fourth most common cause of bloodstream infection and are associated with a dramatically increased mortality as well as increased duration of hospital stay and per patient costs. Besides being a primary

mit klinischer Forschung und einer leistungsfähigen Bioinformatik verknüpft. Weitere Alleinstellungsmerkmale sind die Nutzungsmöglichkeit von Biobanken mit Blut- und Serumproben von Sepsispatienten und die enge Anbindung der Grundlagenforschung an die Klinik. Septomics ist als fakultätsübergreifendes Zentrum der Friedrich-Schiller-Universität Jena (FSU) konzipiert. Wissenschaftlich wird es getragen von der Medizinischen Fakultät bzw. dem Universitätsklinikum über die Klinik für Anästhesiologie und Intensivmedizin (KAI) und dem Institut für Medizinische Mikrobiologie sowie der Biologisch-Pharmazeutischen Fakultät mit dem Leibniz-Institut für Naturstoff-Forschung und Infektionsbiologie (Hans-Knöll-Institut, HKI). Im Gegensatz zum klassischen Bild der „Überaktivierung“ des Immunsystems stellt sich Sepsis in modernen pathophysiologischen Konzeptionen als Ergebnis einer komplexen Dysregulation dar, die sowohl proinflammatorische als auch immuninhibitorische Signale umfasst. Dementsprechend hat sich das Spektrum der Krankheitserreger, die Sepsis in industrialisierten Ländern verursachen, im Laufe der Zeit dramatisch verändert. Dabei sind insbesondere Gram-positive Erreger und Pilze immer wichtiger geworden. In den USA sind Hefepilze der Gattung *Candida* mittlerweile die vierthäufigste Ursache von septischen Infektionen und resultieren daher in beträchtlichen Auswirkungen sowohl im Hinblick auf das therapeutische Outcome der betroffenen Patienten als auch im Hinblick auf Liegedauer und Kosten. Neben primär durch *Candida spp.* verursachten Septitiden kann sich eine invasive Infektion durch *Candida* auch sekundär im Rahmen einer bakteriellen Sepsis oder eines SIRS (severe inflammatory response syndrome) entwickeln. Diese Infektionen resultieren ebenfalls in einer erheblichen Beeinträchtigung der Prognose und deutlich erhöhten Versorgungskosten. Auch andere Pilzinfektionen, insbesondere invasive Aspergillosen treten als

cause for sepsis, invasive candidiasis can also develop as a secondary infection in sepsis (and severe inflammatory response syndrome) which results in deteriorating prognosis and significantly increased health care costs. Infections with other fungal pathogens, mainly invasive aspergillosis, are increasingly diagnosed in severely ill SIRS or sepsis patients and are linked to the immunosuppressive effects of these inflammatory syndromes. Based on these epidemiological developments, the research group fungal septomics has been established within the ZIK Septomics to put a clear focus on the pathophysiology of systemic fungal infections. We have started our work in 11/2009 at the HKI. Within the research concept, molecular mechanisms of pathogen – host interaction resulting in the development of invasivity in *C. albicans* as well as corresponding patterns of immune activation in the context of fungal sepsis will be analysed and compared to similar patterns of other pathogens. The resulting data will improve our understanding of invasive candidiasis. Furthermore they should build a basis for the identification of new diagnostic and therapeutic targets. Those targets may be evaluated within the ZIK Septomics using clinical specimens and the possibility to cooperate with the research group clinical septomics within the centre. Thus it might be possible to develop new diagnostic tests allowing an early diagnosis of sepsis and a discrimination between fungal and bacterial systemic infection.

sekundäre Komplikation bei schwerkranken Sepsis oder SIRS Patienten in zunehmendem Maße auf und sind Ausdruck der aus dem Krankheitsbild resultierenden Immunsuppression. Basierend auf diesen epidemiologischen Entwicklungen wurde mit der FG fungal septomics im ZIK Septomics eine Arbeitsgruppe etabliert, die sich schwerpunktmäßig mit der Pathophysiologie systemischer Pilzinfektionen beschäftigt. Diese Arbeitsgruppe hat zum 1.11.2009 ihre Arbeit am HKI aufgenommen. In dem vorgeschlagenen Projektkonzept der FG Fungal Septomics sollen molekulare Mechanismen der Pathogen – Wirt Interaktion, die zur Entwicklung von Invasivität bei *Candida albicans* führt, und Muster der Immunaktivierung im Rahmen einer Pilzsepsis identifiziert, charakterisiert und mit Mustern bei anderen Erregergruppen vergleichend analysiert werden. Die resultierenden Daten werden zu einem besseren Verständnis der molekularen Mechanismen führen, die der Entwicklung systemischer Infektionen durch *Candida* zu Grunde liegen. Darüber hinaus bilden sie die Basis für translationale Projekte, die zur Entwicklung verbesserter diagnostischer Verfahren sowie zur Identifizierung neuer Ziele für therapeutische Intervention führen sollen. Für neue diagnostische und/oder therapeutische targets, die in diesen identifiziert werden, wird innerhalb des ZIK eine Evaluierung der klinischen Nutzbarkeit anhand von Proben septischer Patienten und durch spätere klinische Studien überprüft werden. Dieses Konzept wird in enger Zusammenarbeit mit der FG Clinical Septomics, die ebenfalls dem ZIK angehört, umgesetzt. Ziel ist die Gewinnung neuer diagnostischer Marker die helfen, Infektionen früh und eindeutig zu diagnostizieren sowie bakterielle Erreger von Pilzen wie *Candida albicans* zu unterscheiden.

1 Summary of prior Research

Group Leader: Oliver Kurzai

Research in the team has focused on molecular and immunological reasons for the accidental establishment of systemic infections caused by normally harmless commensals. *Neisseria meningitidis* and *C. albicans* have been used as model organisms. The Gram-negative diplococcus *N. meningitidis* is a commensal in the nasopharynx of ~10% of healthy individuals which can in rare cases become invasive and cause sepsis or meningitis. Due to its highly toxic lipopolysaccharide, *N. meningitidis* is a prototype cause of sepsis in non-hospitalized patients. We have identified several new strategies of *N. meningitidis* to interact with its host, including a two-partner secretion system, receptors for surface plasminogen recruitment, and a regulatory cascade modulating expression of the major adhesin NadA. A major focus of research has been the recognition of *N. meningitidis* by human DC. We have shown that sialylation and elongation of the meningococcal lipopolysaccharide shields viable unencapsulated *N. meningitidis* from recognition by human DC. The protective function of the LPS for the bacteria can be counteracted *in vivo* by phase variation of the *lgtA* gene encoding LPS glycosyltransferase A. Whereas capsule expression protects *N. meningitidis* efficiently from recognition and phagocytosis by DC independent of the LPS structure, modification of the capsular polysaccharide by O-acetylation did not interfere with recognition, phagocytosis, induction of DC maturation or triggering of cytokine release. The class A scavenger receptor (SRA) could be identified as the major uptake receptor for *N. meningitidis* on human DC (Figure 1). In addition, we have shown that the physical interaction of human DC with *N. meningitidis* via SRA modulates cytokine release by DC. Data from a genome sequencing project on carriage isolates have revealed subtle differences rather than variation in gene content to be responsible for the variable virulence in the species *N. meningitidis* and suggested evolution of pathogenic *Neisseriae* from commensal ancestors. In this context, we characterized the expression and function of the transcriptional regulator FarR (Fatty acid resistance Regulator), a member of the MarR-family, in *N. meningitidis*. In the closely related *Neisseria gonorrhoeae*, FarR is responsible for resistance to long-chained fatty acids, enabling *N. gonorrhoeae* to survive on mucosal surfaces rich of those harmful components. Although the meningococcal FarR-homologue shows high structural accordance, it is not involved in fatty acid resistance. Rather, FarR expression is growth phase dependent and important for immune-evasion of *N. meningitidis*.

For *C. albicans*, major work has dealt with the adaptation of this pathogen to alterations in the ambient pH. This work has led to the description of the Rim101 dependent signalling cascade which mediates pH adaptation and links this environmental stimulus to filamentation of *C. albicans*. Recently, this earlier work on the characterization of morphogenesis related signalling in *C. albicans* has mainly been used as a tool to characterize the interaction of *C. albicans* with human immune cells. Effects of fungal morphogenesis on DC activation were characterized, providing evidence that these cells can recognize yeast and hyphal forms of *C. albicans*. We could show that human neutrophils, which play a major role in the defence against systemic candidiasis, are capable of discriminating between yeast forms and filaments of a single *C. albicans* strain. Whereas germ tubes induced targeted motility, resulting in the establishment of close contact between neutrophils and fungal elements, yeast forms were largely ignored throughout the observation (Figure 2). In transwell chemo-

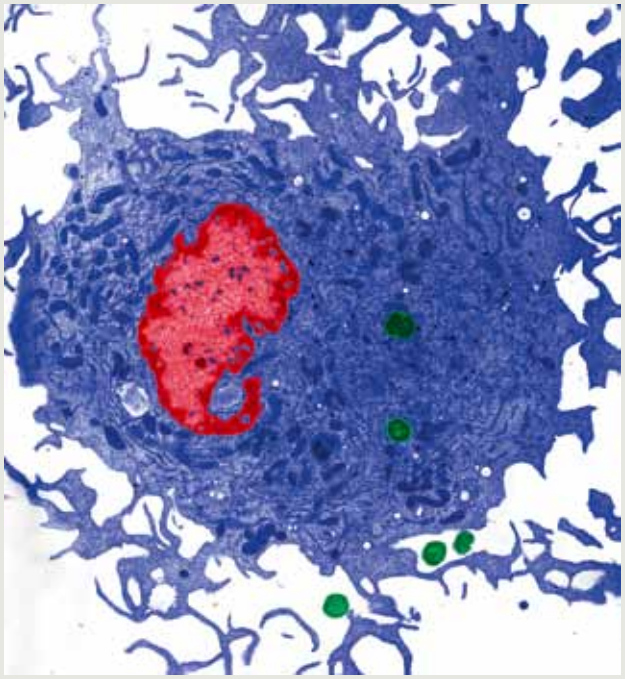

Figure 1
Human dendritic cells are potent antigen presenting cells. For this purpose they possess a variety of surface receptors enabling the uptake of diverse microorganisms. We could identify a member of the Scavenger receptor family (SRA) as the main uptake receptor for *N. meningitidis* on these cells.

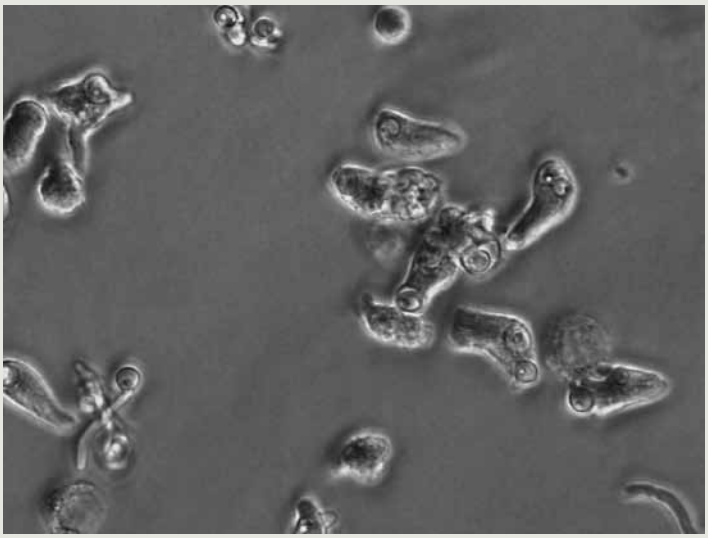

Figure 2
Human neutrophils are activated by filamentous forms of *Candida albicans* and rapidly engulf these forms. Here the intimate contact between these rapidly moving cells and small *C. albicans* germ-tubes is visualized microscopically.

taxis assays, *C. albicans* germ-tubes but not yeast forms induce migratory activity in human neutrophils. In addition filamentous forms triggered a higher oxidative burst than yeast forms of *C. albicans*. In contrast to the oxidative burst however, PMN motility based on actin rearrangement could be shown to be essential for inactivation of *C. albicans* filamentous forms. In contrast, the inhibition of actin reorganization did not impair the ability of human PMN to kill yeast cells. Using inhibitors for different MAP-kinase cascades, it could be shown, that recognition of *C. albicans* filaments by PMN is mediated via the MEK/ERK MAP-kinase cascade and independent of JNK or p38 MAPK activation. Inhibition of the ERK signalling pathway abolished not only neutrophil chemotaxis induced by *C. albicans* filaments but also the ability of human PMN to kill *C. albicans* filaments. In contrast, it did not affect PMN activity against yeast forms. Taken together, these data show that invasive filamentous forms of *C. albicans* trigger a morphotype specific activation of human neutrophils. Therefore these cells are capable of sensing *C. albicans* invasion and intiating an early immune response. In addition, the contribution of secreted aspartic proteases to the interaction of *C. albicans* with human neutrophils (cooperation with Prof. B. Hube, Dept. MPM) and the role of ROI formation in killing of *C. albicans* is subject of ongoing studies. Clinically relevant immunosuppressive agents including mycophenolate (MMF) were investigated for their influence on immune effector cells, demonstrating that MMF leads to reduced secretion of pro-inflammatory cytokines and enhanced production of ROI. In close collaboration with A. Brakhage (Dept. MAM) and J. Löffler (Würzburg), recognition of *A. fumigatus* by the innate immune system has been also been studied in my group.

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Selected publications

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Villwock A, Schmitt C, Schielke S, Frosch M, **Kurzai O** (2008) Recognition via the class A scavenger receptor modulates cytokine secretion by human dendritic cells after contact with *Neisseria meningitidis*. *Microbes Infect* 10, 1158-1165.

Kurzai O, Schmitt C, Friedrich T, Linke B, Vogel U, Frosch M (2008) Whole-genome comparison of disease and carriage strains provides insights into virulence evolution in *Neisseria meningitidis*. *Proc Natl Acad Sci USA* 105, 3473-3478.

Wozniok I, Hornbach A, Schmitt C, Frosch M, Einsele H, **Hube B**, Löffler J, **Kurzai O** (2008) Induction of ERK-kinase signalling triggers morphotype-specific killing of *Candida albicans* filaments by human neutrophils. *Cell Microbiol* 10, 807-820.

Hornbach A, **Heyken A**, **Schild L**, **Hube B**, Löffler J, **Kurzai O** (2009) The glycosylphosphatidylinositol-anchored protease Sap9 modulates the interaction of *Candida albicans* with human neutrophils. *Infect Immun* 77, 5216-5224.

Schielke S, Huebner C, Spatz C, Nägele V, Ackermann N, Frosch M, **Kurzai O**, Schubert-Unkmeir A (2009) Expression of the meningococcal adhesin NadA is controlled by a transcriptional regulator of the MarR family. *Mol Microbiol* 72, 1054-1067.

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Junior Research Group
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Junior Research Group
Microbial Biochemistry and Physiology

Understanding the physiology of fungi during the infection process of human hosts is a prerequisite to efficiently combat severe invasive fungal infections. Without nutrition a pathogen is unable to cause infection and the targeted disruption of the required metabolic pathways may lead to the discovery of new antifungal drugs. Since the metabolic pathways active during the infection process are largely unknown and may vary between species, one of the major aims of the junior research group Microbial Biochemistry and Physiology is the elucidation of the respective fungal metabolic pathways active during infection. Thereby, research is not restricted to primary metabolism but also involves the investigation of secondary metabolites. Most filamentous fungi have a huge capacity to produce various secondary metabolites and some of them are used for human benefits, such as the antibiotic penicillin or the drug lovastatin, which reduces the risk of strokes by lowering the cholesterol level in hypercholesterolemic patients. Contrarily, secondary metabolites may also cause severe mycotoxicoses, increase the risk of cancer and may cause tissue destructions. Since the production of secondary metabolites is strongly dependent on the available nutrients, there is a direct link between primary and secondary metabolism. Therefore, in terms of fungal virulence, both aspects are followed in our research.

To study the establishment and manifestation of fungal infections, suitable model systems are another prerequisite. The most frequently

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Head:
Dr. Matthias Brock

Eine Voraussetzung, um invasive Pilzinfektionen wirksam bekämpfen zu können, ist das Verständnis der Physiologie der Pilze während der Infektionsphase. Ohne eine effiziente Nahrungsaufnahme ist ein pathogener Mikroorganismus nicht in der Lage eine Infektion zu etablieren. Daher könnte die gezielte Unterbrechung des pilzlichen Stoffwechsels zur Entdeckung neuer antifungaler Substanzen führen. Da jedoch die metabolischen Stoffwechselwege, die insbesondere in der Infektionsphase benötigt werden, weitgehend unbekannt sind und diese zwischen verschiedenen Pilzspezies variieren können, ist eine der Hauptaufgaben der unabhängigen Nachwuchsgruppe Mikrobielle Biochemie und Physiologie, die essentiellen Stoffwechselwege für eine Infektionsetablierung aufzuklären. Hierbei wird jedoch nicht der alleinige Fokus auf den Primärmetabolismus gelegt, sondern auch die Produktion und Wirkung sogenannter Sekundärmetabolite untersucht. Die meisten filamentösen Pilze besitzen eine große Kapazität verschiedenste Sekundärstoffe zu produzieren. Durch einige dieser Sekundärmetabolite profitiert die Menschheit in großem Umfang. Beispiele hierfür sind z. B. das Antibiotikum Penicillin oder der Wirkstoff Lovastatin, der das Risiko von Herzinfarkten in Patienten mit Hypercholesterolemie mindert, da er den Cholesterinspiegel senkt. Im Gegensatz hierzu können Sekundärmetabolite von Pilzen jedoch auch schwere Mykotoxikosen verursachen, das Risiko von Krebserkrankungen erhöhen und Gewebsschäden hervorrufen. Da die Produktion dieser Sekundärstoffe meist mit der Verfügbarkeit bestimmter Substrate gekoppelt ist, besteht eine direkte Verlinkung

used model system to study pathogenicity of fungi bases on the infection of immunosuppressed mice. However, to minimise the number of animals used in infection studies, alternative model systems have been developed and compared to the established murine model systems. Besides the use of different cell lines, an embryonated hen's egg infection model seems well suited to study and compare virulence of different fungal strains.

Last but not least, a model system has been established, which allows monitoring of invasive fungal infections by bioluminescence imaging. This system allows visualising the progression of the infection on living animals and will be used to study the efficiency of antifungal treatments in combating disease.

des Primär- und Sekundärmetabolismus. Daher werden in unseren Untersuchungen beide Aspekte betrachtet.

Um die Virulenz von Pilzen untersuchen zu können, sind Infektionsmodelle eine weitere zwingende Voraussetzung. Das meistgenutzte Modellsystem zur Untersuchung der Virulenz filamentöser Pilze basiert auf der Infektion immunsuprimierter Mäuse. Um jedoch die Zahl von Tieren, die in Infektionsstudien eingesetzt werden, zu minimieren, werden alternative Modelle entwickelt und mit dem murinen System verglichen. Neben dem Gebrauch verschiedener Zell-Linien wurde ein embryonisiertes Hühnerei-Modell entwickelt, das gut geeignet erscheint, um die Virulenz verschiedener Pilzstämme miteinander zu vergleichen.

Letztendlich wird seit Kurzem ein Modellsystem eingesetzt, das es erlaubt, den Verlauf invasiver Pilzinfektionen über ein auf Biolumineszenz basierendes Verfahren zu verfolgen. Dieses System erlaubt den Fortschritt einer invasiven Pilzinfektion am lebenden Tier zu verfolgen und wird eingesetzt werden, um die Effizienz einer Behandlung mit antifungalen Substanzen in Infektionsmodellen zu ermitteln und zu optimieren.

1 Propionyl-CoA utilisation in pathogenic fungi

Host proteins may provide one of the main nutrients for fungi during infection. However, the degradation of several amino acids leads to the toxic intermediate propionyl-CoA. In order to avoid the accumulation of this toxic compound, efficient degradation mechanisms need to exist. Previous studies have shown that the filamentous fungus *Aspergillus fumigatus*, which is the main cause of severe invasive aspergillosis, utilises the so-called methylcitrate cycle for propionyl-CoA degradation. A deletion of the gene coding for the key enzyme methylcitrate synthase significantly attenuated virulence. This result confirmed that the fungus consumes indeed nutrients during the infection process, which generate propionyl-CoA.

To check, whether other filamentous fungi with pathogenic potential posses a functional methylcitrate cycle, additional studies were performed on *Fusarium solani* and *Fusarium verticillioides*. All known *Fusarium* strains have the ability to cause plant infections with a broad host spectrum. However, especially *F. solani* has been frequently isolated as the cause of subcutaneous invasive fusariosis. Our investigations revealed that both *Fusarium* species posses a functional methylcitrate synthase, but regulation of cycle activity appeared species specific. *F. verticillioides* displays a high methylcitrate cycle activity in the presence of amino acids, whereas highest activity was observed for *F. solani* in the presence of propionate. Since propionate is a high abundant carbon source in soil, this activity pattern seems to resemble a specific adaptation to the environmental habitat, because *F. solani*, besides being a plant pathogen, is frequently found as a saprophyte in soil. Although further studies need to show, whether the disruption of the methylcitrate cycle attenuates the ability of *Fusarium* species to cause invasive fusariosis, independent investigations revealed that the combined inhibition of the glyoxylate and methylcitrate cycle in *Fusarium graminearum* attenuates plant infection and virulence. Therefore, a role of the methylcitrate cycle in human fusariosis appears rather likely.

Interestingly, although the genomes of most fungi seem to contain genes for a functional methylcitrate cycle, there are some exceptions. One of the most important human pathogenic fungi is the dimorphic yeast *Candida albicans*. A genome search on this fungus revealed no gene coding for any of the methylcitrate cycle enzymes. Additionally, no alternative genes for any other known propionyl-CoA degrading pathway had been identified. Nevertheless, *C. albicans* can utilise proteins as nutrients an can even grow in the presence of propionate or odd-chain fatty acids as sole carbon and energy source (Figure 1). This suggests that *C. albicans* possesses a yet unknown pathway for detoxification and metabolism of propionyl-CoA. Due to the results on other fungi, the targeted disruption of propionyl-CoA metabolism in *C. albicans* could also attenuate virulence. To confirm this assumption and for identifying the responsible pathway in *C. albicans*, metabolites and enzymes are being identified in a JSMC project, which could provide hints for the responsible pathway. Current results imply that *C. albicans* uses a modified beta-oxidation of fatty acids for the metabolism of propionyl-CoA.

2 CoA-transferase for acetic acid detoxification

Soil microorganisms, such as bacteria and fungi, have to cope with rapidly changing environmental conditions, such as temperature and pH differences, water availability, osmotic stress and nutrient supply. Therefore, metabolism requires the adaptation to suboptimal

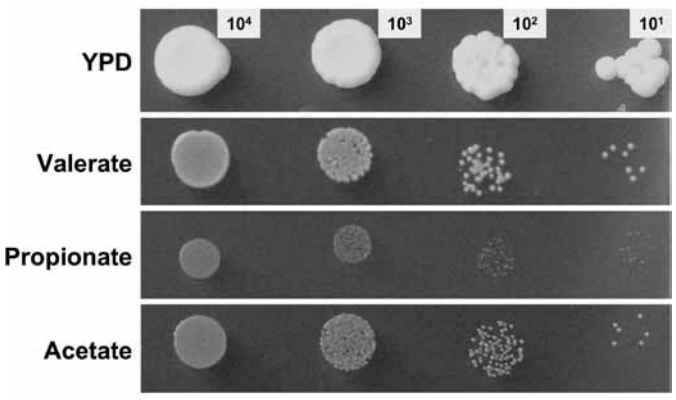

Figure 1
Spot dilution assay for growth determination of *Candida albicans* on media containing different carbon sources. Best growth is observed on the complete medium YPD. Although delayed, cells also grow on minimal media containing the odd-chain fatty acids valerate or propionate and on acetate.

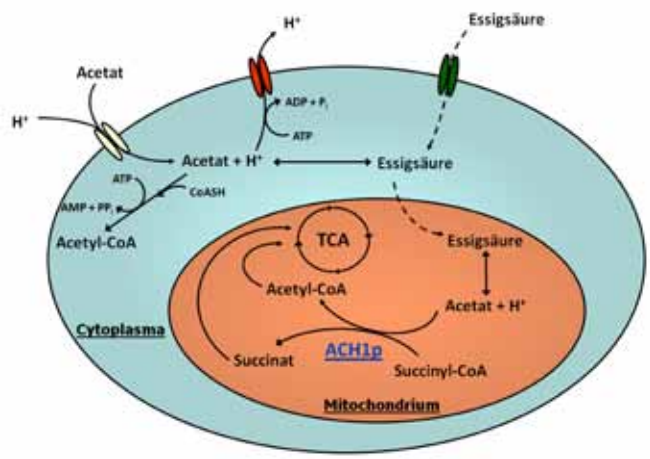

Figure 2
Scheme for the detoxification of mitochondrial acetic acid by the CoA-transferase Ach1p in *Saccharomyces cerevisiae*. Acetic acid can actively be taken up or diffuse over membranes, leading to the acidification of the cytoplasm. A proton export machinery may counteract this acidification. At high influx rates acetic acid can enter the mitochondrial matrix, where it becomes trapped, because activation of acetate to acetyl-CoA is only performed in the cytoplasm. Inside mitochondria the CoA-transferase Ach1p can transfer CoASH from succinyl-CoA to acetate, leading to the citric acid cycle intermediates succinate and acetyl-CoA.

growth conditions. Some acids, such as acetic, propionic, sorbic and benzoic acid can cause a so-called "weak organic acid stress" and an adequate cellular response is required to grow in the presence of these compounds. In our studies we focused on the adaptation of the model fungi *Aspergillus nidulans* and *Saccharomyces cerevisiae* to weak acid stress mediated by acetate at low pH.

Previous studies identified a so-called acetyl-CoA hydrolase Ach1p in *S. cerevisiae*, which caused a growth defect in the presence of acetate when deleted. However, the physiological role of such an energy-wasting enzyme remained a biochemical conundrum. Since we identified a CoA-transferase from *A. nidulans*, which displayed high sequence identity to the yeast Ach1p, we re-characterised the biochemical parameters of the yeast enzyme. Our results showed that also the yeast enzyme acted specifically as a CoA-transferase with highest activity for the transfer of the CoASH-moiety from succinyl-CoA to acetate. The acetyl-CoA hydrolase activity, in contrast, was negligibly low. Additionally, the CoA-transferase CoaT from *A. nidulans* was able to restore growth phenotypes of the yeast mutant on acetate. A more detailed investigation of the growth defect of the mutant on acetate revealed that growth retardation was strongly pH dependent. It could be shown that the lower the pH, the higher the growth inhibitory effect. This implied that Ach1p, and also CoaT in *A. nidulans*, are required for the detoxification of free acetic acid. In the acid form, acetate can freely diffuse over cellular membranes and may finally end up trapped inside mitochondria. Ach1p (or CoaT) can contribute to the detoxification of this organic acid by transferring the CoASH from succinyl-CoA to acetate, forming the citric acid cycle intermediates succinate and acetyl-CoA (Figure 2).

Phylogenetic analyses showed that this enzyme seems conserved in nearly all fungi,

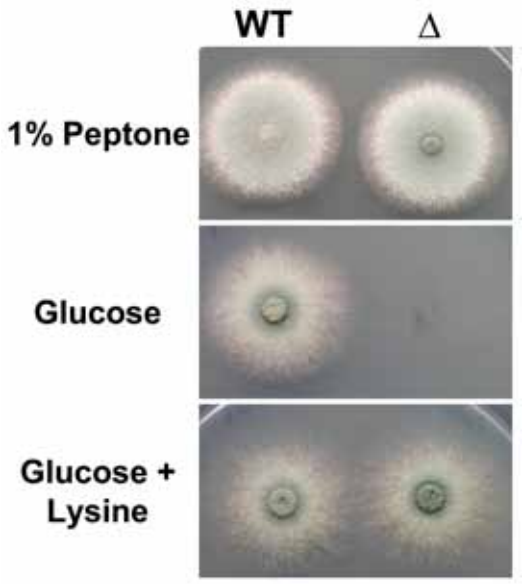

Figure 3
Confirmation of lysine auxotrophy in an *Aspergillus fumigatus* mutant carrying a defective alpha-aminoadipate pathway. Both, wild type (WT) and mutant (Δ) grow well on hydrolysed proteins such as peptone. On minimal media containing glucose as carbon and nitrate as nitrogen source, only the wild type is able to grow. Supplementation of this medium with lysine restores growth of the mutant.

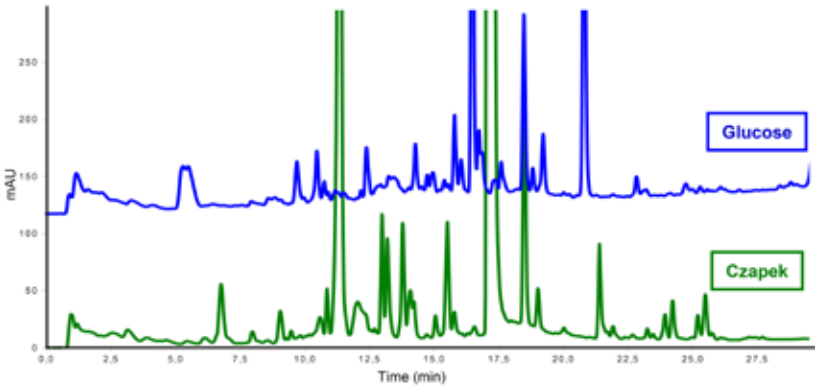

Figure 4
HPLC profiles of medium extracts from *Aspergillus terreus* A1156 grown for 161 h on glucose minimal medium (Glucose) or Czapek dextrose broth (Czapek). A different pool of metabolites is produced in respect to the medium composition.

which implies that the adaptation to acetic acid via the CoA-transferase reaction is an essential prerequisite for fungi in their natural environment.

3 Fungal lysine biosynthesis

Lysine is an essential amino acid for humans and has to be obtained from the diet. However, fungi produce this amino acid via the so-called alpha-aminoadipate pathway. Several studies have focused on the regulation of fungal lysine biosynthesis, because alpha-aminoadipate is an essential precursor for penicillin production in filamentous fungi such as *Penicillium chrysogenum* or *Aspergillus nidulans*. Although the human pathogenic fungus *Aspergillus fumigatus* does not produce this important antibiotic, former studies showed that disruption of the alpha-aminoadipate pathway attenuates virulence in a murine model for invasive bronchopulmonary aspergillosis. Therefore, it has been assumed that the fungal lysine biosynthetic path might provide a suitable target for new antifungals. This prompted us to focus our research on the enzymatic mechanisms of lysine biosynthetic enzymes and to re-evaluate the role of the pathway in virulence using different model systems. The deletion of key genes of the pathway confirmed their essential contribution to lysine prototrophy (Figure 3). Additionally, mutants were unable to germinate on protein containing media unless proteases were added. This shows that the resting conidium is unable to produce proteases for obtaining lysine from protein hydrolysis. In contrast and unexpectedly, lysine auxotrophic mutants were able to grow on blood and serum, indicating that these body fluids contain sufficiently free lysine to support growth. In agreement, an intravenous infection model revealed no attenuated virulence of lysine auxotrophic mutants. Therefore, targeting the lysine biosynthetic path may limit fungal growth within lung tis-

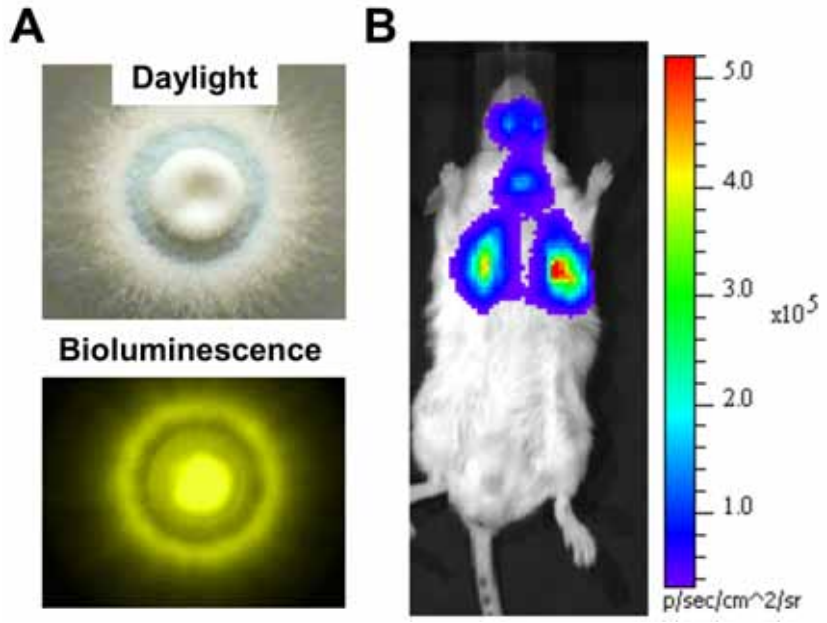

Figure 5
***In vitro* and *in vivo* bioluminescence imaging of a light emitting *Aspergillus fumigatus* strain.**
A: Daylight and bioluminescence photography of a bioluminescent *A. fumigatus* strain on an agar plate containing D-luciferin.
B: Recording of light emission from a mouse infected with the bioluminescent *A. fumigatus* strain shown in (A). After D-luciferin injection the light emission can be detected from all infected organs such as sinus, trachea and lung lobes.

sues, but does not prevent establishment of invasive aspergillosis after dissemination via the bloodstream.

4 *Aspergillus terreus* infections and secondary metabolism

Aspergillus terreus is a potent producer of secondary metabolites and the analysis of its genome implies that only a small number of the metabolites produced by this fungus have been identified. This is mainly due to the tight regulation of secondary metabolite production, which allows the production of certain secondary metabolites only under well-defined conditions (Figure 4). *A. terreus* is especially known for its ability to produce the drug lovastatin, which is a potent inhibitor of the hydroxymethyl-glutaryl-CoA reductase and thus able to lower the amount of cholesterol in patients with hypercholesterolemia. It also produces the metabolite itaconic acid, which is used in the production of plastic, rubber, paints, surfactants and lubricants. However, several other metabolites with less beneficial effects, such as patulin, citroviridin or territremA are produced by *A. terreus*. Similar to *A. fumigatus*, *A. terreus* is able to cause life-threatening invasive aspergillosis in immunocompromised patients and has been identified as an emerging human pathogen. Due to its high resistance against the commonly used antifungal drug amphotericin B the mortality rate among patients with invasive aspergillosis caused by *A. terreus* is up to 100 %. Therefore, the main focus of our research deals with the development of a murine infection model and the elucidation of secondary metabolite production during infection. Murine infection models are performed in close collaboration with the department Microbial Pathogenicity Mechanisms, whereas natural products produced by *A. terreus* are identified and characterised in collaboration with the department Biomolecular Chemistry. First investigations

imply that the strategies of *A. terreus* infecting and colonising a host are slightly different to those from *A. fumigatus*. Nevertheless, the highest risk for obtaining an infection from either species is in a leucopenic model. Recent investigations allowed the identification of a new metabolite, which might be produced even under infectious conditions, but structure elucidation and biological activity of this compound need further studies. However, production of this metabolite requires the presence of amino acids, which is in agreement with the utilisation of host proteins as nutrients. Further investigations will show, whether this metabolite exerts toxic effects and contributes to virulence in the establishment of invasive aspergillosis caused by *A. terreus*.

5 Model systems for virulence studies

Studying virulence determinants essential for the establishment and manifestation of invasive fungal infections requires the use of model systems. The gold standard for such investigations are murine model systems. However, although new biomolecular techniques allow the rapid generation of mutant libraries, not every mutant should become tested in a complicated and time-consuming murine model system. To allow the rapid screening of mutants, we therefore developed together with the department Microbial Pathogenicity mechanisms a new model system using embryonated hen's eggs as recipients. In nature, eggs of birds can become infected with fungal spores through micro cracks in the eggshell and most birds are highly susceptible for *Aspergillus* infections. Our preliminary studies showed that 100 conidia are sufficient to kill the chicken embryo three to six days after infection. Further studies with selected *A. fumigatus* mutants revealed a good correlation between murine model systems and the hen's egg model. Therefore, this model allows the initial screening for attenuated virulence of large amounts of *Aspergillus mutants*.

Another system, just recently developed in collaboration with the Institute Pasteur, uses bioluminescence imaging to follow the manifestation and progression of fungal infections. For this purpose we constructed fungal strains expressing the firefly luciferase under a constitutively active promoter. By that means, a direct correlation between the fungal biomass can be obtained under several *in vitro* conditions, but also during infection under non-inflammatory conditions as present in leucopenic mice (Figure 5). The system allows reproducibly tracking the location of the infection. In first trials we have used the system to follow the effectiveness of an antifungal drug under in vivo conditions. Treatment failure correlated with a strongly increasing bioluminescent signal, whereas a successful treatment resulted in low signals, which disappeared completely, when the infection was eliminated. Further studies will follow improving treatment strategies for combating fungal infections.

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External funding

Deutsche Forschungsgemeinschaft
Excellence Graduate School Jena School for Microbial Communication JSMC
Teilprojekt: Interaction of *Candida albicans* and host cells: Fungal nutrient acquisition and host defense mechanisms
Matthias Brock

Deutsche Forschungsgemeinschaft
Schwerpunktprogramm 1160: Kolonisation und Infektion durch humanpathogene Pilze
Teilprojekt: Carbon-and nitrogen utilisation of pathogenic *Candida* and *Aspergillus* species during pathogenesis
Matthias Brock

Selected publications
(HKI authors in bold)

Ibrahim-Granet O, **Dubourdeau M**, **Latgé JP**, **Ave P**, **Huerre M**, **Brakhage AA**, **Brock M** (2008) Methylcitrate synthase from *Aspergillus fumigatus* is essential for manifestation of invasive aspergillosis. *Cell Microbiol* 10, 134-148.

Fleck CB, **Brock M** (2008) Characterization of an acyl-CoA: carboxylate CoAtransferase from *Aspergillus nidulans* involved in propionyl-CoA detoxification. *Mol Microbiol*. 68, 642-656.

Brock M, **Jouvion G**, **Droin-Bergère S**, **Dussurget O**, **Nicola MA**, **Ibrahim-Granet O** (2008) Bioluminescent *Aspergillus fumigatus*, a new tool for drug efficiency testing and *in vivo* monitoring of invasive aspergillosis. *Appl Environ Microbiol* 74, 7023-7035.

Fleck CB, **Brock M** (2009) Re-characterisation of *Saccharomyces cerevisiae* Ach1p: fungal CoA-transferases are involved in acetic acid detoxification. *Fungal Genet Biol* 46, 473-485.

Brock M (2009) Fungal metabolism in host niches. *Curr Opin Microbiol* 12, 371-376.

Domin N, **Wilson D**, **Brock M** (2009) Methylcitrate cycle activation during adaptation of *Fusarium solani* and *Fusarium verticillioides* to propionyl-CoA generating carbon sources. *Microbiology* 155, 3903-3912.

Collaborations

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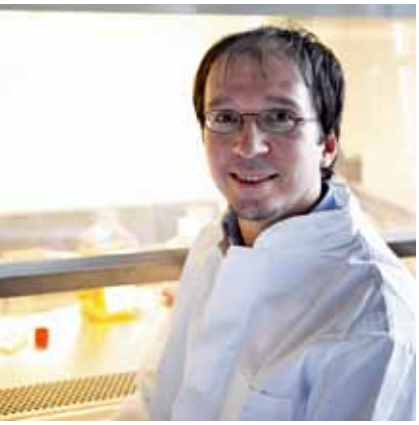

The major role of the immune system is to maintain the integrity of the human body. This in particular implies the ability of the immune system to recognize molecules and cells of our own body as "self", and to detect potentially harmful substances and microorganisms as "non-self". Depending on the nature of the foreign molecule or particle, an immune response to eliminate (the dangerous) or to tolerate/ignore (the non-dangerous) potential threats is initiated. The innate immune system provides an immediately acting, first line of defense against microorganisms. Innate immune cells and molecules are able to sense evolutionary established (i.e., conserved) danger signals and molecular patterns that are associated with the presence of pathogens. These patterns are common in certain pathogen groups and are generally not present in the human body, such as viral or bacterial nucleic acid, bacterial or fungal surface carbohydrate groups, lipopolysaccharides etc. These molecular structures are recognized by soluble (e.g., complement, pentraxins) or membrane bound pattern recognition molecules (such as Toll-like receptors, mannose receptors, beta-glucan receptors) of the host.

Microbes are recognized by immune cells via several mechanisms. One major group of receptors senses molecular patterns associated with the microbes (pattern recognition receptors).

INTRODUCTION | EINLEITUNG

Head:
Dr. Mihály Józsi

Das Immunsystem ist für die Integrität des menschlichen Körpers verantwortlich. Es ist in der Lage, unsere körpereigenen Moleküle und Zellen als „eigen", und potenziell gefährliche Substanzen und Mikroorganismen als „fremd" zu erkennen. Eine effektive Immunantwort wird eingeleitet um gefährliche fremde Partikeln zu eliminieren, während harmlose Moleküle und Mikroorganismen vom Immunsystem toleriert oder ignoriert werden. Das angeborene menschliche Immunsystem ist für die ersten Abwehrreaktionen gegen Mikroorganismen verantwortlich. Zellen und Moleküle der angeborenen Immunität erkennen Bedrohungssignale und molekulare Muster, die mit der Gegenwart von Mikroorganismen assoziiert sind. Diese pathogenassoziierten molekularen Muster, wie z.B. virale Nukleinsäuren, spezielle Kohlenhydratgruppen auf der Oberfläche von Bakterien und Pilzen, sind charakteristisch für bestimmte Gruppen von Mikroorganismen, und kommen in der Regel im menschlichen Körper nicht vor. Diese Muster werden durch lösliche (z. B. Komplement, Pentraxine) und zelluläre Rezeptoren des Wirtes (z. B. Toll-like Rezeptor, Mannoserezeptor, Beta-Glucanrezeptor) erkannt.

Erreger werden durch verschiedene Mechanismen von den Immunzellen erkannt. Eine Gruppe von Rezeptoren erkennt direkt die speziellen molekularen Eigenschaften von Mikroorganismen (Muster-Erkennungsrezeptoren). Eine andere Rezeptorart ist für die Erkennung von pathogengebundenen Wirtsproteinen verantwortlich (sogenannte Opsoninrezeptoren). Die Nachwuchsgruppe Zelluläre Immunbiologie befasst sich mit der Rolle und der Zusammenarbeit dieser verschiedenen

Other receptors bind through host molecules bound on the pathogen (opsonic receptors). The Junior Research Group Cellular Immunobiology studies the role and cooperation of the various receptors and recognition mechanisms in host defense. *Candida albicans* is an opportunistic human-pathogenic fungus which causes severe infections in immunocompromised individuals. In particular, activation of neutrophils and macrophages upon exposure to opsonized and native *Candida albicans* and the role of complement as an immune modulator are analyzed. In addition, we are interested in malfunctions of defense mechanisms and are investigating the role of anti-complement autoantibodies in human diseases.

Rezeptoren des angeborenen Immunsystems bei der Erkennung von Erregern. *Candida albicans* ist ein opportunistischer humanpathogener Pilz, der bei immunsupprimierten Menschen schwere Infektionen verursacht. Wir analysieren insbesondere die zelluläre Antwort von neutrophilen Granulozyten und Makrophagen beim Kontakt mit opsonisierten und nativen *C. albicans*, sowie die Rolle des Komplementsystems in der Wirt-Erreger-Interaktion. Zusätzlich interessieren wir uns für funktionale Defekte in Abwehrprozessen, und untersuchen die Rolle von anti-Komplement Autoantikörpern in humanen Erkrankungen.

1 Interaction of innate immune cells with *Candida albicans*

Phagocytes of the innate immune system are particularly important in antifungal immunity. People who are defective in phagocytic functions due to certain receptor deficiencies, or have low neutrophil counts, are susceptible to fungal infections. Monocytes and neutrophils are circulating in blood; macrophages, dendritic cells and neutrophils act in tissues. Dendritic cells are important as sentinels that sense dangers and alert and activate other immune cells to the presence of pathogens. Neutrophils and macrophages, the major phagocytes of the innate immune system, play an important role in antifungal defense by engulfing and killing pathogens.

Macrophages are phagocytic cells, which reside in tissues throughout the body, and sense invading microbes. Upon activation, they attract additional immune cells, such as neutrophils and lymphocytes, to the site of pathogen entry. Furthermore, as professional phagocytes and antigen presenting cells, they shape the adaptive immune response by activating antigen-specific T cells. Neutrophils are quickly mobilized and migrate into tissues to inflammation sites, where they effectively phagocytose and kill microbes. Macrophages and neutrophils recognize microbes by pattern recognition receptors that bind to specific molecules on microbial surfaces. These receptors mediate phagocytosis and/or initiate various killing mechanisms or the secretion of cytokines and antimicrobial substances.

Several pathogens bind host molecules that help them to evade the immune system. In particular, the acquisition of host complement regulators is a means to escape from the attack of the host complement system. While several bacteria can directly be lysed by complement, pathogens with a thick cell wall are protected from its lytic effect. We have therefore studied whether the human complement regulators factor H and factor H-related protein 1 (CFHR1), when bound on *Candida albicans*, can serve as adhesion ligands for host phagocytic cells. We found that factor H and CFHR1 can bind to *C. albicans* and also to human neutrophil granulocytes. A neutrophil cell surface molecule, which is an integrin receptor composed of two chains ($\alpha_M\beta_2$ integrin, also known as CD11b/CD18), was identified as the major receptor for factor H and CFHR1. Factor H and CFHR1, when bound on *C. albicans*, supported neutrophil migration and enhanced adhesion/phagocytosis of the yeasts by neutrophils. In addition, the release of the antimicrobial protein lactoferrin, and the production of reactive oxygen species by neutrophils was enhanced by factor H and CFHR1. The enhanced physical contact and antimicrobial activity in the presence of these two proteins resulted in increased killing of the pathogen (Figure 1). Thus, the two host proteins factor H and CFHR1, when bound on the surface of *C. albicans*, mediate host-pathogen interaction, enhance the antimicrobial activity of human neutrophils and lead to a more efficient fungal killing. Since various pathogens have been shown to bind factor H, and macrophages and dendritic cells also express CD11b/CD18, this mechanism may apply to other cases of host cell-pathogen interaction as well.

Fungi display on their surface and also secrete molecules that function as immunomodulators by activating or inhibiting host responses. Pra1p (pH-regulated antigen 1) has been identified in *C. albicans* as a surface protein, which is highly expressed and is secreted by *C. albicans* hyphae. The receptor for Pra1p on neutrophils and macrophages is the same CD11b/CD18 molecule, which binds factor H and CFHR1. Thus, Pra1p likely plays

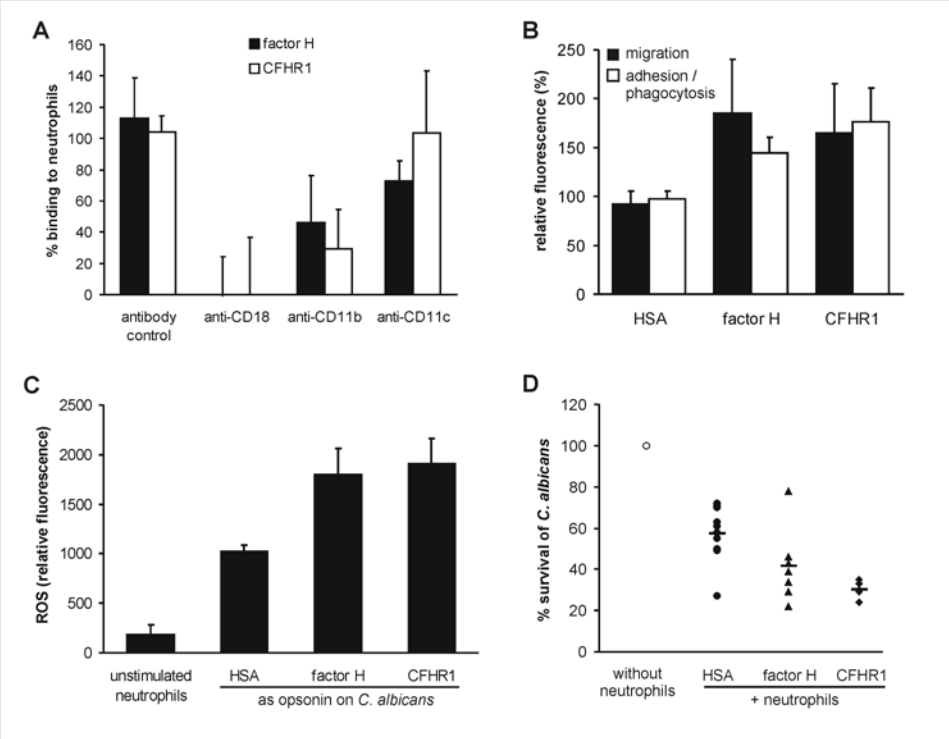

Figure 1
Interaction of *Candida albicans* with human neutrophils is enhanced by complement molecules of the host innate immune system. (A) The two related complement proteins factor H and factor H-related protein 1 (CFHR1) bind to neutrophil granulocytes via the CD11b/CD18 cell surface receptor. (B) Factor H and CFHR1, when bound to *C. albicans*, support the migration of neutrophils, and enhance adhesion/phagocytosis of yeast cells by neutrophils. HSA: human serum albumin, used as a control protein. (C) Factor H and CFHR1 increase the production of reactive oxygen species (ROS) by neutrophils upon contact with *C. albicans*. (D) Yeast-bound factor H and CFHR1 promote host-pathogen interaction and lead to a more efficient fungal killing by human neutrophils. Data points represent independent experiments with neutrophils derived from various blood donors.

a role in host cellular responses to different morphological forms of the fungus. In this project, the potential immunomodulatory effects of Pra1p on human neutrophils and macrophages is investigated. In cooperation with the Department of Microbial Pathogenicity Mechanisms, we are studying mutant *C. albicans* strains lacking or overexpressing Pra1p, in comparison with the wild-type strain, for functional effects on human phagocytic cells, such as adhesion and migration of phagocytes, and the release of antimicrobial substances and cytokines upon exposure to the pathogen.

2 Crosstalk between pentraxins and the complement system

Pentraxins are soluble recognition molecules of the innate immune system. They are characterized by a common pentraxin domain and occur in multimeric, most often pentameric form. The major pentraxins are the short pentraxins C-reactive protein (CRP) and serum amyloid P protein, and the long pentraxin PTX3. The prototypic short pentraxin CRP is produced in the liver and is a commonly used inflammatory marker because its concentration is increased up to 1000-fold in human plasma after trauma or infections, e.g. during sepsis. CRP is a pattern recognition molecule that binds to several ligands exposed on microbes and on damaged host cells, such as phosphocholine, phosphoethanolamine, histones and chromatin. CRP can also bind to phagocytes and thus promotes removal of certain microbes and altered host cells. In addition, CRP activates the host complement system, which leads to deposition of complement proteins on the surface where CRP is bound. This process, when foreign or modified self particles (such as apoptotic and necrotic cells) are marked with host molecules in order to facilitate recognition and uptake by phagocytes, is called opsonization.

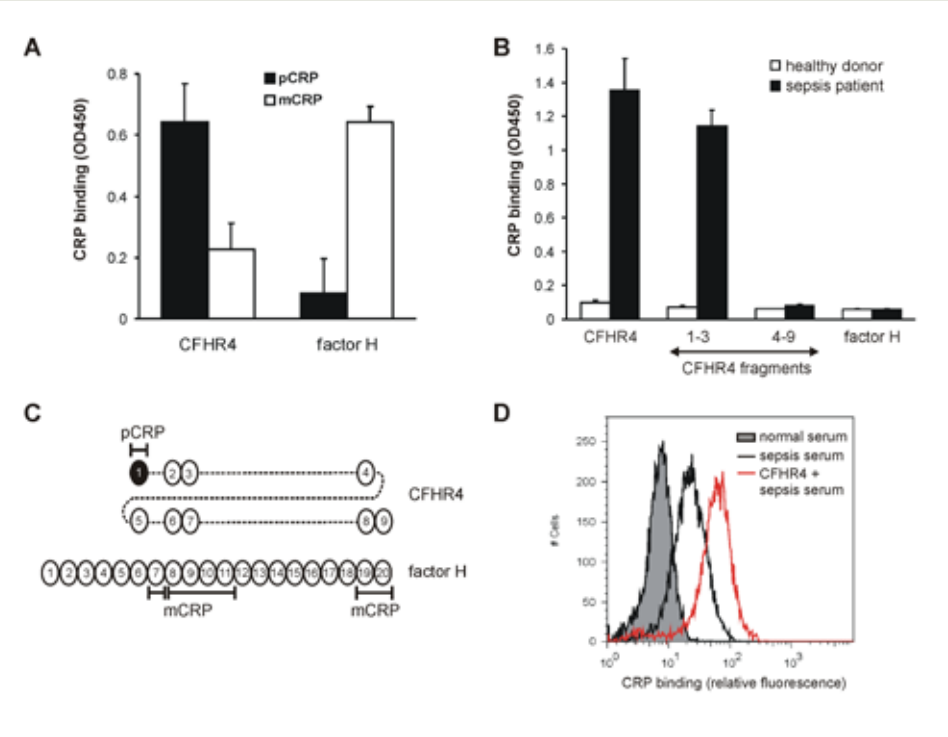

Figure 2
Factor H-related protein 4 (CFHR4) enhances opsonization through its interaction with the pentraxin C-reactive protein (CRP). (A) The two closely related complement proteins CFHR4 and factor H bind to different forms of CRP. While CFHR4 primarily interacts with native, pentameric CRP (pCRP), which is the CRP form that is found in high concentrations in plasma after infections (e.g., in patients with sepsis), factor H binds mainly to denatured, monomeric CRP (mCRP). (B) Native CRP binds from plasma of sepsis patients to the N-terminal fragment (domains 1-3) of CFHR4. (C) The figure shows the 9 domains of CFHR4 vertically aligned with the homologous domains of factor H. The pCRP binding site was localized to domain 1 of CFHR4 using peptide arrays and mutation analysis. In contrast to this, factor H interacts with mCRP via three binding sites unrelated to the pCRP binding domain of CFHR4, indicating a functional specialization among these related proteins. (D) CFHR4 binds to damaged host cells, such as necrotic cells, and recruits native CRP from sepsis serum, thus enhancing opsonization. This may facilitate removal of dangerous self by opsonophagocytosis.

Major opsonins of the innate immune system include various complement components and proteins of the pentraxin family.
Complement activation in the host, however, when not appropriately regulated, can cause severe tissue damage. It was shown that the complement regulator factor H can bind to CRP, thus it was speculated that while CRP initiates opsonization, an over-activation of complement is prevented by the simultaneous binding of the inhibitor factor H. In this project, in collaboration with the Department of Infection Biology, we aimed to characterize the interaction of CRP with factor H.

Native CRP is composed of five identical subunits that build up a pentameric structure and is stabilized by calcium. A denatured, monomeric CRP form can be generated *in vitro*, e.g. by Ca^{2+}-deprivation, chemical or physical denaturation, but its physiological role is unclear. When analyzing the interaction of factor H with the two CRP forms, we found that under physiological conditions, i.e. at low CRP levels, factor H primarily interacts with denatured CRP. This interaction of the two proteins was found to be relevant on the surface of damaged cells, where CRP could recruit factor H, which resulted in enhanced phagocytosis of apoptotic cells by macrophages.

Factor H belongs to a family of closely related proteins, which share several homologous domains with each other. Recently, we have cloned a long isoform of human factor H-related protein 4 (CFHR4), which has 9 domains related to certain domains of factor H. Because of this structural similarity, we hypothesized that CFHR4 is also a CRP binding protein. Indeed, CFHR4 bound to native, but not to denatured, CRP already at low concentrations, in sharp contrast to factor H. CFHR4 and CRP could be co-precipitated from the serum of patients suffering from sepsis, indicating relevance of this interaction during pathophysiological conditions. In addition, CFHR4 bound

to necrotic cells and was able to recruit CRP to the necrotic cell surface (Figure 2). This could lead to enhanced opsonophagocytosis and removal of damaged cells from tissues.

We have further analyzed the molecular basis and the functional consequence of the CFHR4-CRP interaction. Fragments of CFHR4, representing different parts of the molecule, were recombinantly generated. Among these fragments, only the one containing the first domain of the molecule bound native CRP (Figure 2). Peptide array experiments identified residues 35-41 of CFHR4 to be involved in CRP binding. Mutation analyses confirmed that these amino acids are important for the interaction. We could also show that CRP, when bound to CFHR4, can activate complement and results in C3 fragment deposition. Mutant CFHR4 proteins with reduced CRP binding capacity induced less complement activation. Sequence comparison with related domains of other factor H-related proteins and analysis of related peptides for CRP binding indicated that the CRP binding motif in CFHR4 is unique among these closely related proteins. In summary, we showed that factor H and CFHR4 preferentially interact with different CRP forms. The data reveal the molecular basis of the specific interaction of CFHR4 and native CRP, and suggest a role of CFHR4 in enhancing opsonization via CRP binding. We propose that by this mechanism CFHR4 regulates complement activation and opsonization on microbes and altered host cells and thus contributes to their safe clearance from the body.

The long pentraxin PTX3 is produced locally by innate immune cells and was shown to play a role in antifungal defense. The aim of this project is to study the interactions between PTX3 and the complement system and the role of this cooperation in host defense, especially in the opsonophagocytosis of microbes. This project is financially supported in part by the Jena School for Microbial Communication (JSMC).

3 Anti-complement autoantibodies in kidney diseases

Complement is an ancient and promptly acting defense system of innate immunity, which recognizes and eliminates microorganisms, initiates inflammation and also modulates adaptive immune responses. Complement is composed of ca. 35 components which are present in body fluids and on cell membranes. Activation of this protein network is initiated by target recognition via three activation cascades, which merge in a common terminal pathway and can lead to the lysis of microbes. However, at the same time complement activation is controlled by both fluid phase and cell membrane-anchored regulators in the host in order to prevent collateral tissue damage. Several diseases are associated with defective complement regulation, including the kidney diseases atypical hemolytic uremic syndrome (aHUS) and dense deposit disease.

In aHUS patients mutations have been identified in complement genes, and these mutations affect the balance of complement activation and inhibition. Recently, an acquired, autoimmune form of aHUS, characterized by circulating autoantibodies against the complement regulator factor H, has been described. Our aim is to understand the role of anti-factor H autoantibodies in aHUS. In collaboration with the Department of Infection Biology and with clinicians, we are analyzing how autoantibodies affect the physiologic function of factor H and thus lead to disease.

Factor H is a plasma complement regulator, which is built up from 20 globular domains. The four N-terminal domains are responsible for the complement inhibitory activity of factor H. The two C-terminal domains are critical for the recognition and binding to host cell surfaces, thus factor H protects host cells from complement-mediated damage. In order to assess the effect of the autoantibodies on factor H functions, we first determined the binding sites of the autoantibodies. To this end, recombinant factor H fragments and various monoclonal antibodies that bind to different domains of factor H were used. All

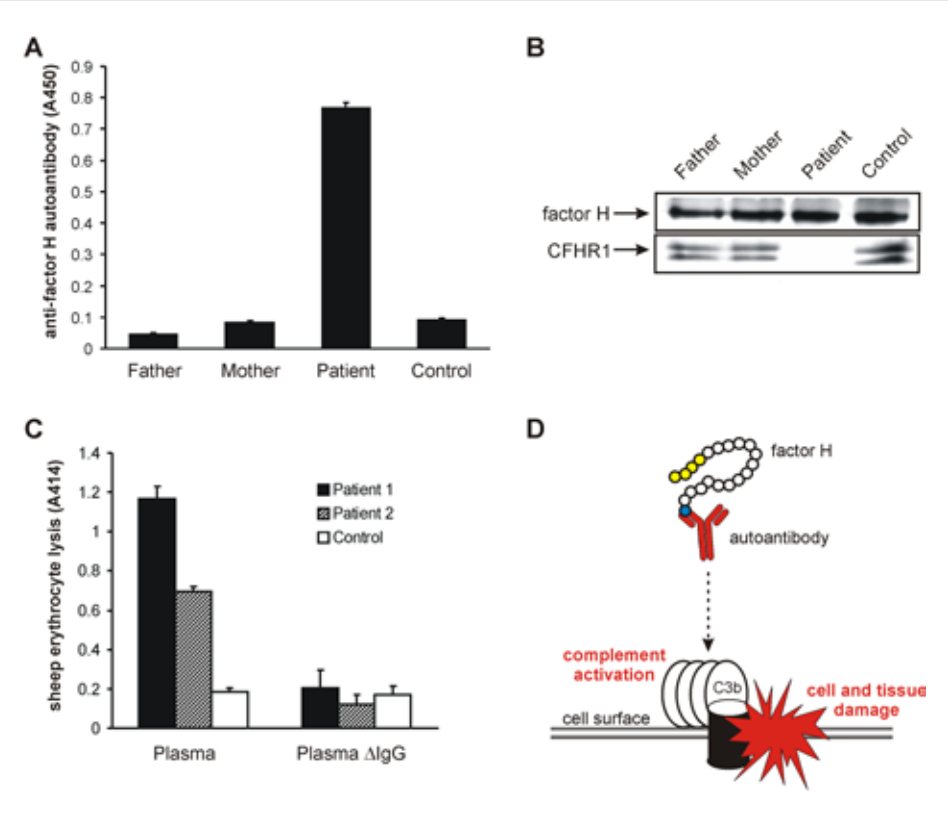

Figure 3
Role of anti-factor H autoantibodies in atypical hemolytic uremic syndrome.
(A) The acquired form of the kidney disease atypical hemolytic uremic syndrome is characterized by the presence of circulating autoantibodies against the complement regulator factor H. (B) The majority of the patients with anti-factor H autoantibodies has a genetic defect leading to the loss of the *CFHR1* gene; consequently, the two isoforms of the CFHR1 protein are not detectable in the patients' plasma by Western blot. (C) Plasma of the patients cause anomalous lysis of sheep erythrocytes in an *in vitro* model of host cell protection. When the autoantibody-containing IgG fraction is removed from plasma (Plasma ΔIgG), the host cells are rescued from complement-mediated lysis. (D) Autoantibodies bind to the most C-terminal domain 20 of factor H (blue), which is responsible for host cell and ligand recognition. Our results show that the autoantibodies inhibit factor H binding to host cells, so that it cannot regulate complement locally (mediated by the complement regulatory domains of factor H, shown in yellow). This leads to enhanced complement activation on the cell surface and causes complement-mediated cell damage.

analyzed autoantibodies (n=23) bound to the most C-terminal domain of factor H, suggesting that the autoantibodies interfere with the host cell recognition function of this complement inhibitor. Functional assays demonstrated that the autoantibodies indeed inhibit factor H binding and its protective activity on host cells. Plasma samples of aHUS patients were analyzed in an *in vitro* cell protection assay using sheep erythrocytes as a model for host cells. Sheep erythrocytes, similar to human cells, have polyanionic molecules on their surface, which allow binding of factor H from human plasma. Thus, in normal human plasma sheep erythrocytes are not lysed by complement. Plasma samples of patients with aHUS, however, caused enhanced hemolysis of sheep erythrocytes. This anomalous lysis could be reversed by adding purified factor H, i.e. by an excess of antibody-free, functional protein. Similarly, when the IgG fraction was removed from these plasma samples, no anomalous lysis was observed, providing direct evidence of factor H-function blocking autoantibodies in the patients (Figure 3). This was confirmed by analyzing patients' plasma samples containing different amounts of autoantibodies: we found that the autoantibody levels show an inverse correlation with factor H cell surface activity.

These findings are being translated into specific treatment, including plasma exchanges, to remove the antibodies and provide functional factor H, and immunosuppressive therapy to inhibit autoantibody production. The disease activity and the autoantibody levels are monitored in the patients during treatment, which is important in order to assess the efficacy of the applied therapies.

In addition, the development of these autoantibodies is genetically predisposed: in the majority of the patients the factor H-related gene *CFHR1* is missing (Figure 3). Therefore, we analyze the role of the CFHR1 protein in

developing autoantibodies and whether autoantibodies against other complement proteins exist in these patients. Furthermore, by measuring autoantibodies in other kidney diseases, we address the question whether anti-factor H autoantibodies are specific to aHUS. Preliminary data from the analysis of plasma samples from patients with dense deposit disease, a kidney disorder also associated with impaired complement regulation, revealed no anti-factor H antibodies, but in some cases autoantibodies against the complement protein factor B. We are currently analyzing the role of these new autoantibodies in dense deposit disease.

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Role of factor H autoantibodies in hemolytic uremic syndrome and other kidney diseases
Mihály Józsi

Selected publications

(HKI authors in bold)

Józsi M, Licht C, **Strobel S**, Zipfel SL, **Richter H**, **Heinen S**, **Zipfel PF**, **Skerka C** (2008) Factor H autoantibodies in atypical hemolytic uremic syndrome correlate with CFHR1/CFHR3 deficiency. *Blood* 111, 1512-1514.

Józsi M, **Zipfel PF** (2008) Factor H family proteins and human diseases. *Trends Immunol* 29, 380-387.

Mihlan M, **Hebecker M**, **Dahse HM**, **Hälbich S**, Huber-Lang M, Dahse R, **Zipfel PF**, **Józsi M** (2009) Human complement factor H-related protein 4 binds and recruits native pentameric C-reactive protein to necrotic cells. *Mol Immunol* 46, 335-344.

Mihlan M, **Stippa S**, **Józsi M**, **Zipfel PF** (2009) Monomeric CRP contributes to complement control in fluid phase and on cellular surfaces and increases phagocytosis by recruiting factor H. *Cell Death Differ* 16, 1630-1640.

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Junior Research Group
Fundamental Molecular Biology of Pathogenic Fungi

Junior Research Group
Fundamental Molecular Biology of Pathogenic Fungi

Only a little number of fungal species share the ability to induce infections in humans, including the yeast *Candida albicans* as well as dermatophytes. Addressing this peculiarity, the Junior Research Group Fundamental Molecular Biology of Pathogenic Fungi set out to further elucidate the molecular basis of the biology and pathogenicity of these fungi. The opportunistic pathogen *C. albicans* is a frequent and harmless commensal on mucosal surfaces of the gastrointestinal and urogenitary tract in healthy people. In the immunocompromised host however, *C. albicans* can cause superficial as well as life-threatening disseminating infections. Important for multiplication and propagation of the microorganism during disease appears to be its high flexibility. Most notably to name is the ability of *C. albicans* to rapidly adapt to changing environmental conditions, the tolerance to diverse stresses, and the capacity to switch between different morphologies. Upon distinct environmental parameters, *C. albicans* can not only grow as round-oval budding yeasts, but also in form of long filaments and large, spherical chlamydospores. In our studies we identify and functionally analyse individual *C. albicans* genes which are putatively important for the above mentioned, particular characteristics of the pathogen. In addition, we investigate communication strategies of *C. albicans* and elucidate their potential influence on host adaptation and pathogenicity.

INTRODUCTION | EINLEITUNG

Head:
Dr. Peter Staib

Nur wenige Pilze lösen Infektionen bei Menschen aus, darunter der Hefepilz *Candida albicans* sowie Hautpilze. Um die Besonderheit dieser Erreger besser zu verstehen, werden in der Nachwuchsgruppe Molekularbiologische Grundlagen pathogener Pilze grundlegende Mechanismen ihrer Biologie und Pathogenität erforscht. *C. albicans* ist bei vielen Menschen als harmloser Kommensale auf Schleimhäuten des Verdauungs- und Urogenitaltraktes zu finden, kann jedoch bei Abwehrgeschwächten oberflächliche sowie lebensbedrohliche, disseminierende Infektionen hervorrufen. Der Erreger kann sich gut an unterschiedliche Umweltbedingungen anpassen und erträgt verschiedenste Stressfaktoren. Diese Merkmale erscheinen wichtig für Vermehrung und Ausbreitung von *C. albicans* bei einer Infektion. Vielseitig zeigt sich auch die Gestalt des Pilzes, die in Abhängigkeit verschiedenster Umweltparameter wechseln kann zwischen ovaler, knospender Hefezelle, Filament und kugelförmiger Chlamydospore. In unseren Studien identifizieren und analysieren wir individuelle *C. albicans* Gene, die für diese Leistungen wichtig sind. Darüberhinaus beleuchten wir Kommunikationsstrategien des Pilzes und deren möglichen Einfluß auf Wirtsadaptation und Pathogenität.

Hautpilzinfektionen, sogenannte Dermatophytosen, sind nicht lebensbedrohlich, dafür bei Mensch und Tier sehr weit verbreitet und oft schwer zu therapieren. Die Pathogenität von Dermatophyten, einer spezialisierten Gruppe filamentöser Pilze, ist bislang weitgehend un-

Infections by dermatophytes, so called dermatophytoses, are not life threatening, however, they are widespread and in many cases difficult to cure. The pathogenicity of dermatophytes, a specialized group of filamentous fungi, is so far poorly understood. In addition, only a little number of tools are available for the molecular analysis of these microorganisms, which grow comparatively slowly under *in vitro* conditions. For a better understanding of their pathogenicity, we address the question, why these fungi exclusively infect host structures containing the hard, compact protein keratin: Skin, hair and nails. Using the zoophilic dermatophyte species *Arthroderma benhamiae* as a model we have identified not only factors which are putatively involved in the growth of the pathogen on such substrates under *in vitro* conditions, but also during infection in an animal model. For subsequent functional analysis of the candidate dermatophyte genes we established a genetic system in *A. benhamiae* which allows the specific deletion of individual genes.

Our basic research should contribute to a better understanding of the pathogenicity of *C. albicans* and dermatophytes, and should offer ideas for novel antifungal targets in the development of future therapeutic strategies.

verstanden. Zudem stehen zur molekularen Analyse dieser im Labor langsam wachsenden Pilze auch nur wenige Werkzeuge zu Verfügung. Eine zentrale Frage zur Pathogenität von Dermatophyten besteht darin, warum diese Erreger nur Wirtsstrukturen befallen, die das schwer degradierbare Eiweiß Keratin enthalten: Haut, Haare und Nägel. Am Beispiel der zoophilen Spezies *Arthroderma benhamiae* prüfen wir nicht nur, welche Faktoren der Pilz einsetzt, wenn er unter Laborbedingungen auf solchen Nährstoffen wächst, sondern auch während der Infektion im Tiermodell. Zur funktionalen Analyse der auf diesem Wege identifizierten Faktoren haben wir ein genetisches System in *A. benhamiae* entwickelt, mit dessen Hilfe individuelle Gene spezifisch deletiert werden können.

Durch unsere Grundlagenforschung soll letztlich nicht nur die Pathogenität von *C. albicans* sowie die von Hautpilzen besser verstanden werden, sondern möglicherweise auch neue Zielstrukturen zur Entwicklung neuer Therapieansätze entdeckt werden.

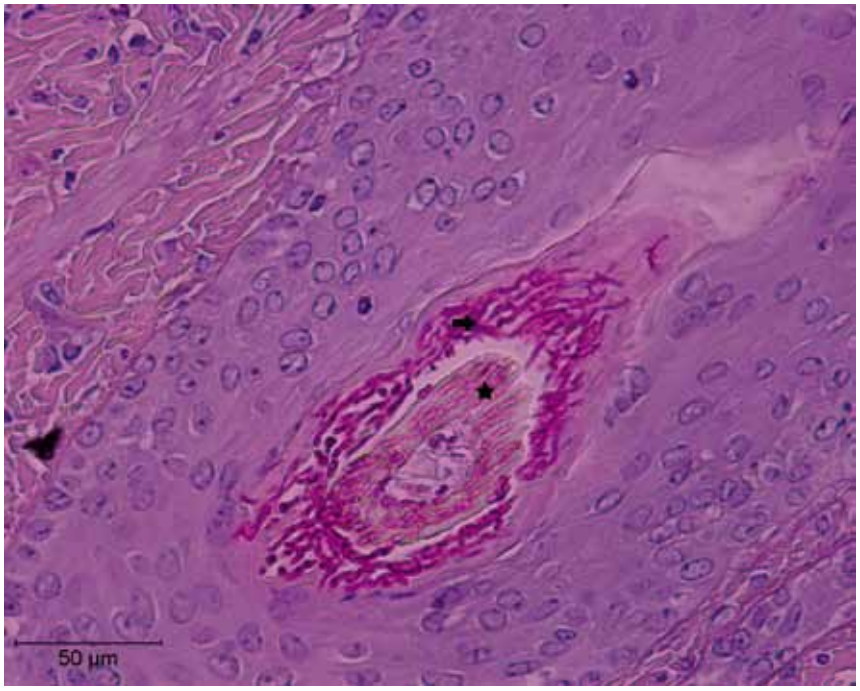

Figure 1
Cutaneous guinea pig dermatophytosis 11d post infection with *A. benhamiae*. PAS-stained cross-section microscopy of an infundibular-isthmal hair visualizes *A. benhamiae* cells colonizing the hair cortex (star) and hair shaft (arrow). The scale bar represents 50 µm. (Staib et al., 2009)

1 Identification of putative pathogenicity mechanisms in dermatophytes

In vitro digestion of keratin by dermatophytes is associated with the secretion of multiple proteases, which are assumed to be responsible for their particular specialization to colonize and degrade keratinized host structures during infection. To investigate the role of individual secreted proteases in keratin degradation, a cDNA microarray was developed for *Trichophyton rubrum*, the clinically most prevalent dermatophyte species (Zaugg *et al.*, 2009). The array covers approximately 20–25 % of the genome and contains sequences of at least 23 protease genes. Gene expression profiles during growth of *T. rubrum* on soy and keratin protein displayed the activation of a large set of genes encoding secreted endo- and exoproteases. In addition, other specifically induced factors with potential implication in protein utilization were identified, including heat shock proteins, transporters, metabolic enzymes, transcription factors and hypothetical proteins with unknown function.

In order to get a better understanding of the pathogenicity of dermatophytes we further focussed our research on the zoophilic dermatophyte *Arthroderma benhamiae*. *A. benhamiae* causes highly inflammatory cutaneous infections in humans and rodents, and provides basic characteristics making the species an adequate model to study the pathogenesis of dermatophytes. Compared to the related species *T. rubrum*, it is relatively fast growing on standard laboratory media, produces abundant microconidia and is able to undergo sexual reproduction. The genome sequence of the *A. benhamiae* strain which we use in our studies has become available since recently (A. Brakhage, HKI, unpublished results), and a guinea pig infection model for this fungus was developed in our work (Figure 1). By microarray analysis we revealed an *in vivo* pro-

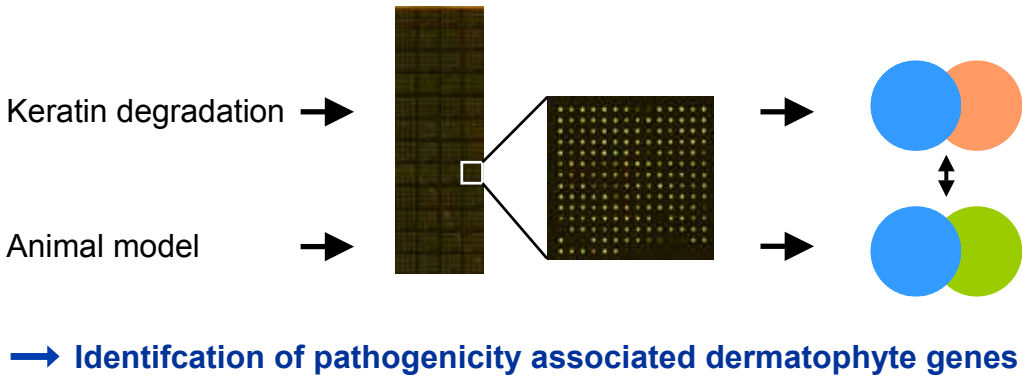

Figure 2
Dermatophyte genes which are putatively associated with the degradation of keratin and/or infection of guinea pigs were identified by broad-scale microarray analysis.

tease gene expression profile in *A. benhamiae* cells, which was surprisingly different from the pattern elicited during *in vitro* growth on keratin (Figure 2, Staib *et al.*, 2009). Instead of the major *in vitro* expressed proteases, e.g. subtilisin 3 and 4 as well as metalloproteases 3 and 4 and leucine aminopeptidases, others were activated specifically during infection. These enzymes were therefore suggested to fulfill important functions that are not exclusively associated with the degradation of keratin. Of particular interest, the gene encoding the serine protease subtilisin 6, a known major allergen in *T. rubrum* and putatively linked to host inflammation, was found as the most strongly upregulated gene during infection. In addition, other candidate pathogenicity related factors were identified, such as genes encoding key enzymes of the glyoxylate cycle, an opsin-related protein, multiple transporters and hypothetical proteins. This work provided to our knowledge the first broad scale gene expression profile in human pathogenic dermatophytes during infection and points to putative virulence associated mechanisms that make these microorganisms the most successful etiologic agents of superficial mycoses.

2 Isogenic strain construction and gene targeting in pathogenic dermatophytes

In general, filamentous fungi are known to only poorly support the specific integration of linear DNA cassettes in the genome by homologous recombination. However, in different filamentous fungi an increased frequency of targeted insertion has been detected in mutants which lack enzymes of the Ku recombinase complex, e.g. in *Neurospora crassa*, *Aspergillus fumigatus*, and since recently also in the dermatophyte *Trichophyton mentagrophytes*. Nevertheless, using such mutants as parental strains for isogenic strain construction and subsequent mutant analysis it has

References

Staib P, Zaugg C, Mignon B, Weber J, Grumbt M, Pradervand S, Harshman K, Monod M (2010) *Microbiology* 156, 884–895.

Rennemeier C, Frambach T, Hennicke F, Dietl J, Staib P (2009) *Infect Immun* 77, 4990–4997.

Zaugg C, Monod M, Weber J, Harshman K, Pradervand S, Thomas J, Bueno M, Giddey K, Staib P (2009) *Eukaryot Cell* 8, 241–250.

to be taken into consideration, that absent Ku activity can lead to secondary, unforeseen genetic alterations and phenotypes. Therefore, in the first line, we set out to delete candidate genes in our *A. benhamiae* wild-type strain. To achieve this, a transformation protocol was established which allows efficient integration of a linear DNA cassette at the target locus, using a hygromycin or neomycin resistance gene as a selection marker after transformation. As an example, the *A. benhamiae* gene encoding the putative malate synthase was deleted by this strategy. Given the possibility that specific deletion of one or the other gene in *A. benhamiae* wild-type cells might not be as efficient, we also constructed a mutant in the *A. benhamiae KU70* locus. Using this mutant as a parental strain for genetic manipulation, an increased frequency of targeted mutagenesis by homologous recombination was observed. Notably, the *ku70* mutant strain showed similar growth phenotypes under the tested *in vitro* conditions as compared to the wild type. In a next step, wild type and *ku70* mutant strains will also be compared for their virulence potential during guinea pig infection. These experiments are necessary for future analyses to prove whether the lack of *KU70* has any effect on the virulence of *A. benhamiae* in our infection model. In conclusion, we can offer a straightforward genetic system in *A. benhamiae*, a prerequisite for future functional genetics in this pathogen.

3 The role of distinct metabolic pathways in dermatophytes for substrate adaptation and pathogenicity

Keratin and lipids are major constituents of the skin. During experimental infection of guinea pigs by *A. benhamiae* and subsequent transcriptional profiling we detected an up-regulation of fungal genes encoding key enzymes of the glyoxylate cycle, i.e. the putative malate synthase and isocitrate lyase. Based on this observation and the knowledge from other microorganisms that this metabolic pathway is involved in the utilization of lipids, we addressed the question whether these factors are involved in dermatophyte pathogenicity. For this approach, *A. benhamiae* mutants in the *AcuE* gene (encoding the malate synthase A) were constructed and tested for their ability to grow on different substrates *in vitro*. Hereby, we found that growth of these mutants was impaired on media with lipids as the sole carbon source. In a next step, wild type and mutant strains will be tested for their virulence during *in vitro* infection of reconstituted human epidermis, during cocultivation with phagocytes and also during experimental guinea pig infection. Since mutants in *AcuE* were not only generated in the wild type, but also in the *A. benhamiae ku70* mutant background, both sets of mutants will be analysed during infection.

4 The sulfite efflux pump Ssu1 has diverse functions in *Candida albicans*

Sulfite is commonly added as a preservative during the production of wine, due to its antimicrobial and antioxidative properties. Therefore, the molecular basis of sulfite tolerance has been intensively studied in fermenting yeasts and led to the identification of the sulfite efflux pump Ssu1 in *Saccharomyces cerevisiae*. In our work we investigated the functional role of the putative sulfite efflux pump Ssu1 in the human pathogenic yeast *Candida albicans*. We discovered that mutants lacking this transporter are not only growth sensitive to toxic sulfite, but also to nitric oxide and cysteine. The inhibitory effect of elevated cysteine concentrations on the growth of *C. albicans ssu1* mutants was not only revealed during yeast cell growth, but also during the formation of hyphae. Elevated levels of this sulfur containing amino acid have long been known to be growth inhibiting for a variety of prokaryotic and eukaryotic cells, yet the molecular basis of cysteine tolerance and toxicity remained mostly obscure. Notably, we found that the *C. albicans SSU1* gene is strongly induced during growth of the fungal cells specifically by cysteine (Fig.3), but not by cystine, methionine or sulfite, which is a metabolite of cysteine. In addition, we confirmed the previous finding that the *SSU1* gene is also induced by nitric oxide, and interestingly, in

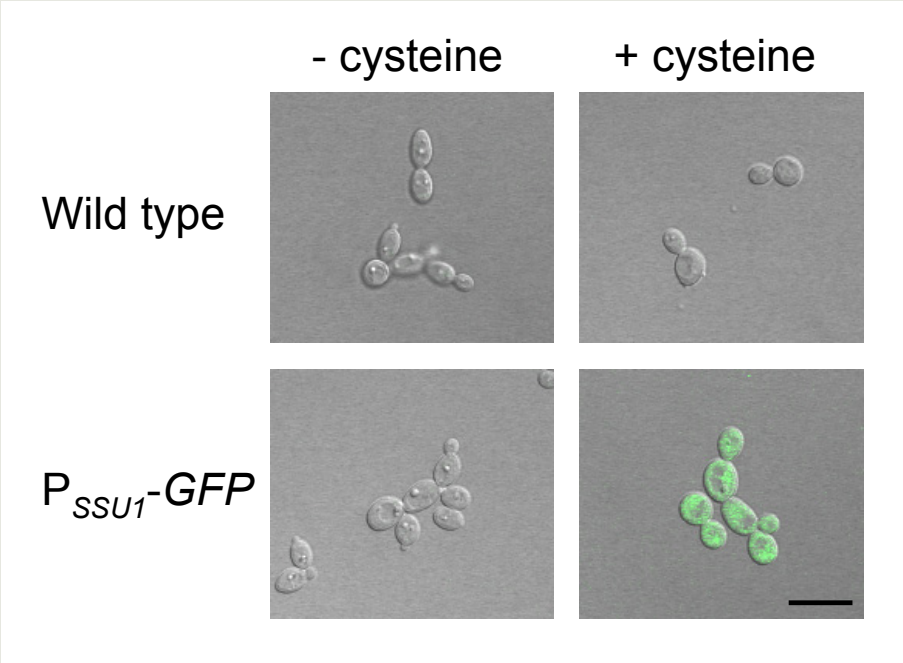

Figure 3
The *C. albicans SSU1* gene is induced in the presence of cysteine. *C. albicans* cells of the wild type SC5314 and P_{SSU1}-*GFP*-reporter strain were grown for six hours in synthetically defined medium in the absence (left) or presence (right) of 5 mM L-cysteine. The reporter strain contains the *GFP* gene (encoding the green fluorescent protein) under control of the promoter P_{SSU1} in one of the two *SSU1* alleles. Photographs were taken by fluorescence microscopy. The scale bar represents 10 µm.

our hands *C. albicans ssu1* mutants were also growth sensitive in the presence of a nitric oxide donor. Our findings on Ssu1 give the first insights into the molecular basis of cysteine tolerance in *C. albicans* and further suggest multiple functions of this transporter in this pathogenic yeast. At present, we further investigate the complex mode of transcriptional regulation of sulfite, cysteine and nitric oxide tolerance in *C. albicans*.

5 Microbial communication and host interaction

The phenomenon of quorum sensing has received particular attention not only in the study of microbial communication within bacterial populations, but also in interkingdom signaling and pathogenicity. Quorum sensing (QS) is defined as a tool of microorganisms to sense their population density by the release of signaling molecules, to which they in turn respond. Reaching a threshold level in a microbial population, these molecules can regulate diverse effects including bioluminescence, biofilm formation and virulence gene expression. Various QS-molecules have been described for many bacterial species such as the autoinducing peptides or N-acyl-homoserine lactones. A eukaryotic QS-system was first identified in *C. albicans*. The isoprenoid alcohol farnesol was shown to inhibit the transition of *C. albicans* yeast cells to filamentous growth forms, and an opposite effect was found for tyrosol. Both these molecules are detectable in the supernatant of *C. albicans* yeast cell cultures in micromolar amounts.

A potential adverse influence of microorganisms on human gametes has so far only rarely been investigated, although infertility in men and women is in many cases associated with genital contaminations by various commensal or uropathogenic microbes. Given that many microorganisms are known to re-

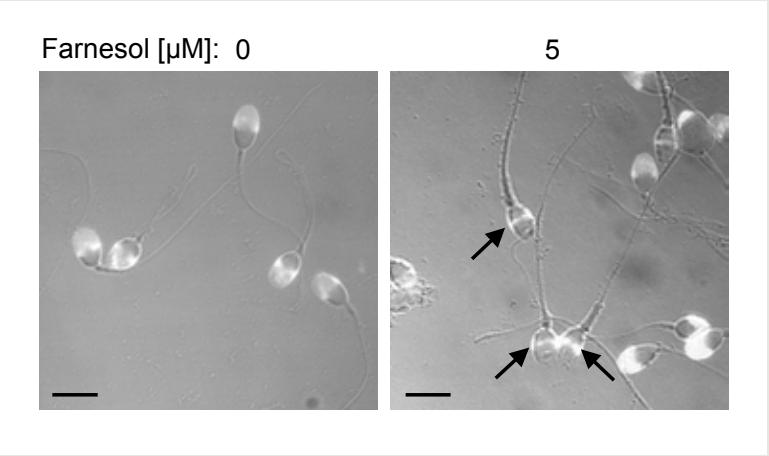

Figure 4
Farnesol treatment results in acrosome loss in human spermatozoa. Spermatozoa were treated for one hour with 5 µM farnesol. The sperm acrosomes were detected by fluorescence microscopy using FITC-conjugated *Pisum sativum* agglutinin which binds to intact acrosomal membranes. Intact acrosomes were identified by a uniform fluorescence in the acrosomal region of the sperm head, whereas acrosome loss was revealed by absent fluorescence or equatorial segment staining (arrows). The scale bars represent 5 µm. (Rennemeier *et al.*, 2009)

lease QS-signals in substantial amounts, we raised the question whether such molecules can directly affect human spermatozoa. In our work we found that farnesol and 3-oxododecanoyl-L-homoserine lactone employed by *C. albicans* and the gram negative bacterium *Pseudomonas aeruginosa*, respectively, induce multiple damages in spermatozoa (Rennemeier *et al.*, 2009). A reduction in the motility of spermatozoa coincided dose-dependently with apoptosis and necrosis at molecule concentrations which were non-deleterious for dendritic-like immune cells. Of particular interest, sublethal doses of both signaling molecules induced a premature loss of the acrosome, a cap-like structure of the sperm head which is essential for fertilization (Figure 4). Addressing their mechanism of action, we discovered that the bacterial molecule, but not the fungal substance, actively induced the acrosome reaction in spermatozoa via a calcium dependent mechanism. This work uncovered a new facet in the interaction of microorganisms with human gametes and thereby suggested a putative link between microbial communication systems and host infertility. In addition, we show that spermatozoa offer an interesting model in the analysis of host pathogen interaction.

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Excellence Graduate School Jena School for Microbial Communication JSMC
Teilprojekt: Analysis of environmental signalling and morphogenetic control in the human pathogenic yeast *Candida albicans*
Peter Staib

Selected publications

(HKI authors in bold)

Degel B, **Staib P**, Rohrer S, Scheiber J, Martina E, Büchold C, Baumann K, Morschhäuser J, Schirmeister T (2008) Cis-configured aziridines are new pseudo-irreversible dual-mode inhibitors of *Candida albicans* secreted aspartic protease 2. *ChemMedChem* 3, 302-315.

Staib P, Lermann U, Blaß-Warmuth J, Degel B, Würzner R, Monod M, Schirmeister T, Morschhäuser J (2008) Tetracycline-inducible expression of individual secreted aspartic proteases in *Candida albicans* allows isoenzyme-specific inhibitor screening. *Antimicrob Agents Chemother* 52, 146-156.

Zaugg C, Jousson O, Léchenne B, **Staib P**, Monod M (2008) *Trichophyton rubrum* secreted and membrane-associated carboxypeptidases. *Int J Med Microbiol* 298, 669-682.

Zaugg C, Monod M, Weber J, Harshman K, Pradervand S, Thomas J, Bueno M, Giddey K, **Staib P** (2009) Gene expression profiling in the human pathogenic dermatophyte *Trichophyton rubrum* during growth on proteins *Eukaryot Cell* 8, 241-250.

Rennemeier C, Frambach T, **Hennicke F**, Dietl J, **Staib P** (2009) Microbial quorum sensing molecules induce acrosome loss and cell death in human spermatozoa. *Infect Immun* 77, 4990-4997.

Staib P, Zaugg C, Mignon B, Weber J, **Grumbt M**, Pradervand S, Harshman K, Monod M (2010) Differential gene expression in the pathogenic dermatophyte *Arthroderma benhamiae in vitro* versus infection. *Microbiology* 156, 884-895.

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Junior Research Group
Secondary Metabolism of Predatory Bacteria

Junior Research Group
Secondary Metabolism of Predatory Bacteria

For decades, predatory behaviour was thought to be restricted to eukaryotic organisms, and bacteria were only granted a role as a nutrient resource for larger sized hunters, such as amoeba and nematodes. Today we know that predatory bacteria are widespread in nature and that they can be retrieved from a multitude of soil and aquatic habitats. Albeit their population size rarely reaches an immoderate level, it is far from negligible. Predatory bacteria have influenced and still shape the structure of microbial communities. They may also have a profound impact on the evolution of their prey. Their ecological significance notwithstanding, we know surprisingly little about these organisms. Some species live primarily as saprophytes, and only certain, largely unexplored factors trigger predatory behaviour. The ability to excrete lytic enzymes allows them to feed, among other substrates, on a wide array of dead and living bacteria (Figure 1). In contrast, a second group consists of obligate predators. While the latter are mostly solitary hunters, the former have learned to work cooperatively in a team. Upon the detection of potential prey, single cells recruit numerous conspecifics using a sophisticated system of intercellular communication. In consequence, further micropredators gather and draw close to their victims. The concerted secretion of cell wall degrading enzymes facilitates the enzymatic lysis of prey cells and thus benefits every member of the pack.

INTRODUCTION | EINLEITUNG

Head:
Dr. Markus Nett

Jahrzehntelang ging man davon aus, dass sich räuberische Verhaltensweisen nur in eukaryotischen Lebewesen manifestieren und dass Bakterien allenfalls als Nahrungsgrundlage für größere Jäger wie Amöben und Nematoden dienen. Heute wissen wir, dass räuberische Bakterien weitverbreitet in der Natur vorkommen und in einer Vielzahl von Boden- und aquatischen Habitaten nachgewiesen werden können. Obschon ihre Populationsdichte nur selten übermäßig hohe Werte annimmt, ist sie doch weit davon entfernt, dass man sie vernachlässigen könnte. Räuberische Bakterien wirken sich auf die Zusammensetzung von mikrobiellen Gemeinschaften aus. Vermutlich haben sie auch maßgeblich die Evolution ihrer Beute beeinflusst. Trotz ihrer ökologischen Signifikanz wissen wir überraschend wenig über diese Organismen. Einige Arten leben überwiegend als Saprophyten, und nur bestimmte, größtenteils nicht erforschte Faktoren lösen ein prädatorisches Verhalten aus. Die Fähigkeit, lytische Enzyme abzusondern, ermöglicht ihnen sich zusätzlich zu anderen Substraten von einer Vielzahl von toten und lebenden Bakterien zu ernähren (Abb. 1). Eine zweite Gruppe besteht demgegenüber aus obligat lebenden Räubern. Während letztere zumeist einzelgängerisch vorgehen, haben die Erstgenannten gelernt, im Team zu jagen. Nach der Entdeckung potentieller Beute rufen einzelne Zellen zahlreiche Artgenossen herbei unter Nutzung eines ausgeklügelten, interzellulären Kommunikationssystems. In der Folge versammeln sich weitere der kleinen Räuber und nähern sich ihren Opfern. Die gemeinschaftliche Freisetzung von Zellwand-zersetzenden Enzymen erleichtert die enzymatische

Our group is mainly interested in the biosynthetic pathways that have evolved in these bacteria. One major incentive to explore the secondary metabolism of wolfpack feeding bacteria comes from recent genome sequencing projects, which have shown that their chromosomes are particularly rich in genes encoding the production of natural products. Still, we can only speculate about a functional role for many of these compounds. As secondary metabolism is generally assumed to enhance the competitiveness of bacteria, it is reasonable to predict that at least some of the natural products are part of the predatory strategy and help to paralyse or kill the prey. Besides, many low-molecular metabolites act as global regulators within microbial communities ena-

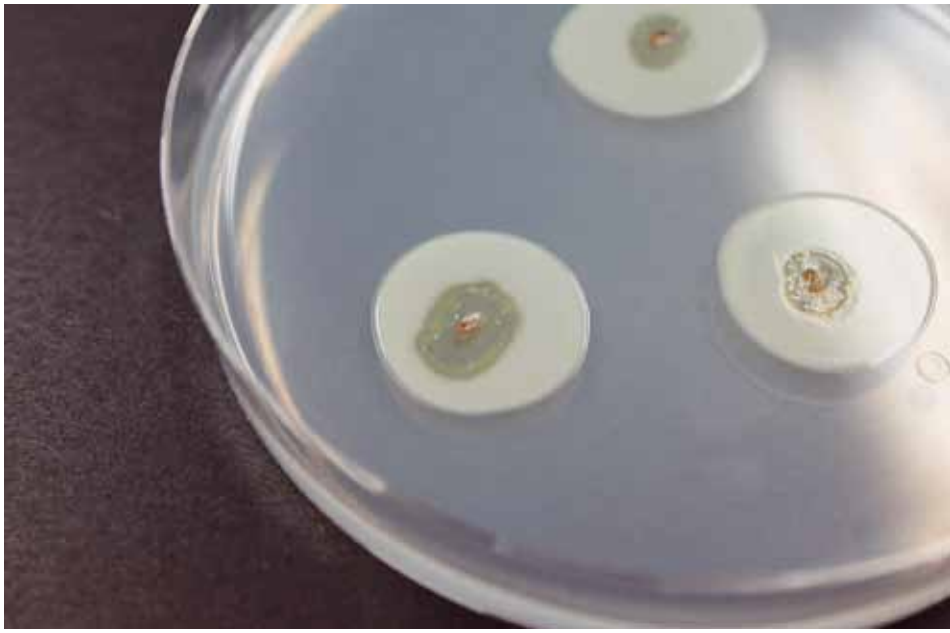

Figure 1
Spots of living *E. coli* cells are lysed by predatory bacteria.

Auflösung der Beutezellen und kommt damit jedem Mitglied des Rudels zugute.

Unsere Gruppe interessiert sich in erster Linie für die Biosynthesewege, die in diesen Bakterien im Verlauf der Evolution entstanden sind. Ein wesentlicher Anreiz für die Erforschung des Sekundärstoffwechsels kommt von Sequenzierungsprojekten, die darauf hindeuten, dass die Genome von in Rudeln jagenden Bakterien besonders reich an Genen sind, die für die Produktion von Naturstoffen kodieren. Noch immer können wir über eine Funktion für viele dieser Verbindungen nur spekulieren. Es wird allgemein angenommen, dass der Sekundärmetabolismus die Durchsetzungsfähigkeit von Bakterien steigert. Im Zusammenhang mit räuberischen Bakterien liegt daher nahe, dass zumindest einige der Naturstoffe Bestandteil der Jagdstrategie sind und zur Lähmung oder Tötung der Beute eingesetzt werden. Darüber hinaus wirken viele niedermolekulare Verbindungen als globale Regulatoren innerhalb mikrobieller Gemeinschaften und ermöglichen eine komplexe Kommunikation. Fragen, die wir zu lösen versuchen, sind: Welche Verbindungen werden von Wolfsrudel-Bakterien produziert? Wie werden diese Stoffe gebildet? Ist ihre Synthese eine Voraussetzung für räuberisches Verhalten und zu welchem Zweck werden sie produziert?

Die Beantwortung dieser Fragen erfordert eine multi-disziplinäre Herangehensweise, die Methoden aus den Bereichen Bioinformatik, Molekularbiologie, Biochemie und Chemie mit einschließt. Über eine Homologie-basierte

bling a complex communication. Questions that we are trying to resolve include: Which compounds are produced by wolfpack feeding bacteria? How are these compounds made? Is their production a prerequisite for predatory behaviour and what function do they serve?

To this end, we are applying a multidisciplinary approach that integrates methods of bioinformatics, molecular biology, biochemistry and chemistry. Homology-driven genome analysis is used to identify loci of putative biosynthetic pathways. Transcription analyses are then key to determine the fermentation conditions under which the respective clusters are expressed. Once a promising gene cluster has been detected it is possible to predict the chemical structure of the encoded natural product and to screen for its physicochemical or pharmacological properties in fermentation studies. In cases where the cluster remains silent under laboratory culture conditions we are transferring the entire pathway into a different bacterial host in order to achieve expression. By engineering knockout mutations in the biosynthetic loci we interrogate their function and learn about the role of natural products in predation.

Genomanalyse werden mögliche Biosynthese-Loci zunächst identifiziert. Mittels Transkriptionsanalysen werden dann die für eine Expression erforderlichen Kultivierungsbedingungen bestimmt. Nachdem ein vielversprechendes Gen-Cluster gefunden wurde, kann die chemische Struktur des darauf kodierten Naturstoffes vorhergesagt werden. Über die physikochemischen oder pharmakologischen Eigenschaften kann so gezielt in Fermentationsstudien nach der betreffenden Verbindung gesucht werden. Im Fall eines sog. stillen Clusters übertragen wir den vollständigen Stoffwechselweg in ein anderes Bakterium um eine Expression zu erreichen. Indem wir Knockout-Mutationen in den Biosynthese-Loci generieren, können wir gezielt untersuchen, welche Rolle die betreffenden Naturstoffe für die Jagd auf Beuteorganismen spielen.

1 Novel peptide antibiotics from rational genome mining

Genomic analyses have illuminated the secondary metabolic proficiency of microbes – underappreciated for years based on conventional isolation programs – and have helped set the foundation for a new natural product discovery paradigm based on genome mining. Biosynthetic genes are in general successively arranged into operon-like clusters that very often include additional regulatory elements and resistance mechanisms. In the case of polyketide synthase and nonribosomal peptide synthetase assembly lines, the identity and sequence of protein domains determines the number and order of building blocks incorporated into the natural product as well as tailoring reactions. By means of computational sequence comparison tools and biosynthetic precedence the structures of many previously unobserved metabolites can thus be predicted, which in turn allows the development of suitable fermentation and genetic methods to activate or enhance their production.

In a model study we scanned the genome of the wolfpack feeding bacterium *Herpetosiphon aurantiacus* for genes involved in secondary metabolism. This study led to the discovery of multiple biosynthetic loci, many of which encode the synthesis of peptidic natural products. To our own surprise, three distinct gene clusters were predicted to incorporate the non-proteinogenic amino acid 4-hydroxyphenylglycine (4-HPG) into their respective products. 4-HPG arises from the diversion of the tyrosine degradation pathway into secondary metabolism and its biosynthesis requires a set of three enzymes. The corresponding gene cassette is widely spread in actinomycete bacteria, yet, it has never been reported from another taxonomic group of eubacteria, including *H. aurantiacus* and its relatives. Even though we detected putative copies of all three 4-HPG encoding genes on the *H. aurantiacus* chromosome, the homologies of the deduced proteins were rather low when compared to their actinobacterial counterparts. At this point, it was unclear whether the sequence deviations were due to a phylogenetic bias or whether they indicated a different function. Therefore, we reconstituted the entire pathway in vitro, which unequivocally confirmed that *H. aurantiacus* has the genetic potential to produce 4-HPG (Figure 2). Subsequent investigations of the bacterial transcriptome by RT-PCR implied that the 4-HPG pathway is coordinately expressed along with the aforementioned biosynthetic gene clusters under select fermentation conditions. Since the lack of a genetic system in *H. aurantiacus* impeded a knockout study to trace the 4-HPG containing natural products, we envisaged a genomisotopic approach to guide the isolation of the corresponding compounds. For this purpose, a select building block is fed to the bacterium in an isotopically labelled form. Metabolites incorporating the labelled precursor can then be tracked in the fermentation broth by selective NMR experiments. We assumed 4-HPG to be highly suited for this type of study, as its non-proteinogenic nature prevents a high metabolic turnover. The chemical synthesis of ^{15}N-labelled 4-HPG was recently accomplished and the feeding study is currently in progress.

2 Total enzymatic synthesis

One strategy to unravel the products of orphan pathways is to reconstitute the entire biosynthesis in vitro. We were pursuing the latter approach in case of an orphan type-III polyketide synthase (PKS) gene that was detected during our functional annotation of the *H. aurantiacus* genome and lacks any homologs in phylogenetically related bacteria, raising questions about its function. In contrast to type-I and type-II PKSs, the type-III PKSs consist of small, ho-

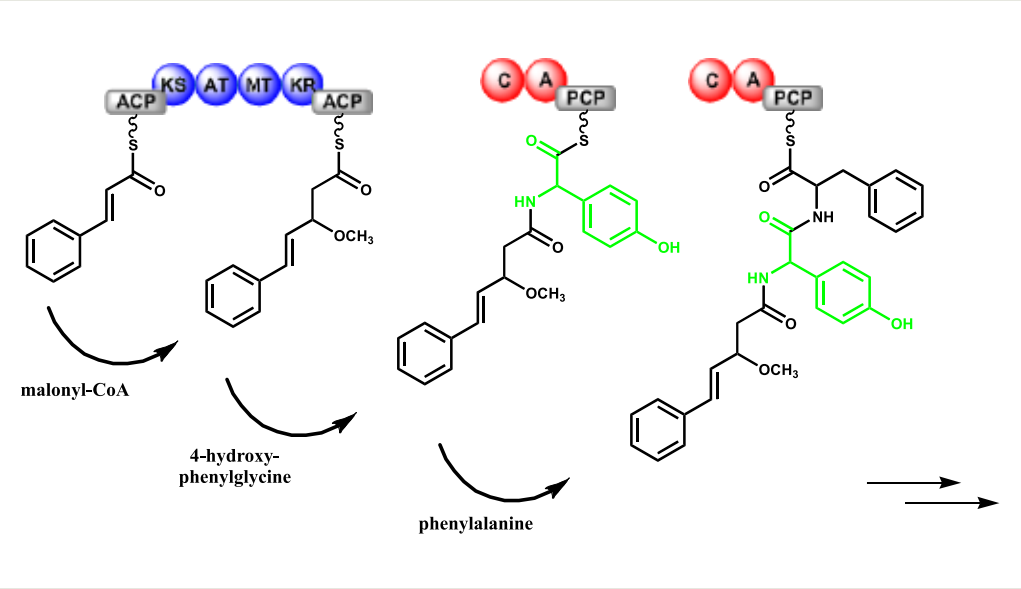

Figure 2-1
Partial biosynthetic assembly line encoded on the *H. aurantiacus* genome. The non-proteinogenic amino acid 4-hydroxyphenylglycine (highlighted in green) is incorporated into the growing polyketide/peptide chain.

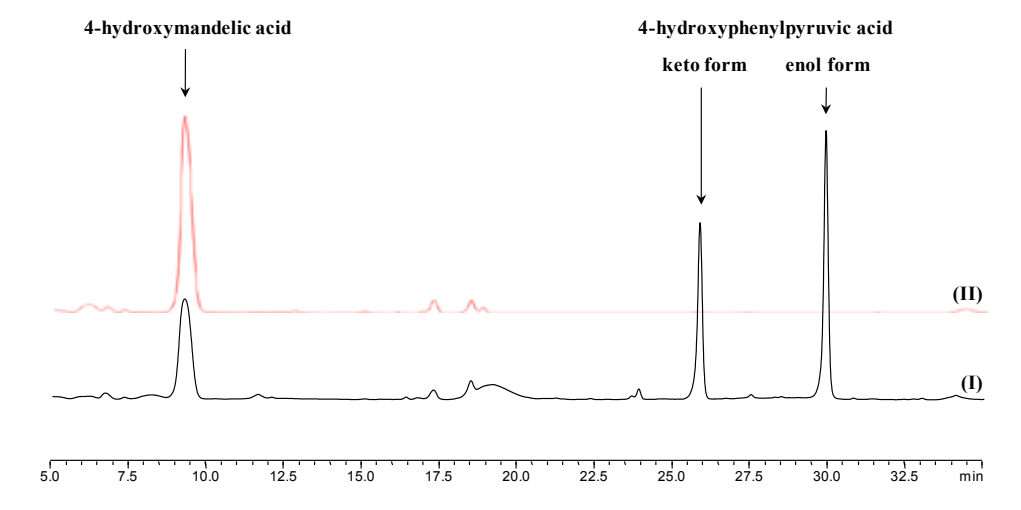

Figure 2-2
HPLC trace showing (I) the enzymatic conversion of 4-hydroxyphenylpyruvic acid into 4-hydroxymandelic acid, a key step in the biosynthesis of 4-hydroxyphenylglycine, and (II) authentic D,L-4-hydroxymandelic acid.

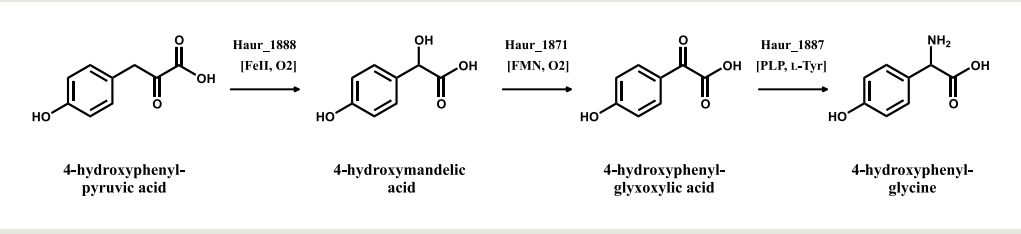

Figure 2-3
Reconstituted pathway to 4-hydroxyphenylglycine from *H. aurantiacus*.

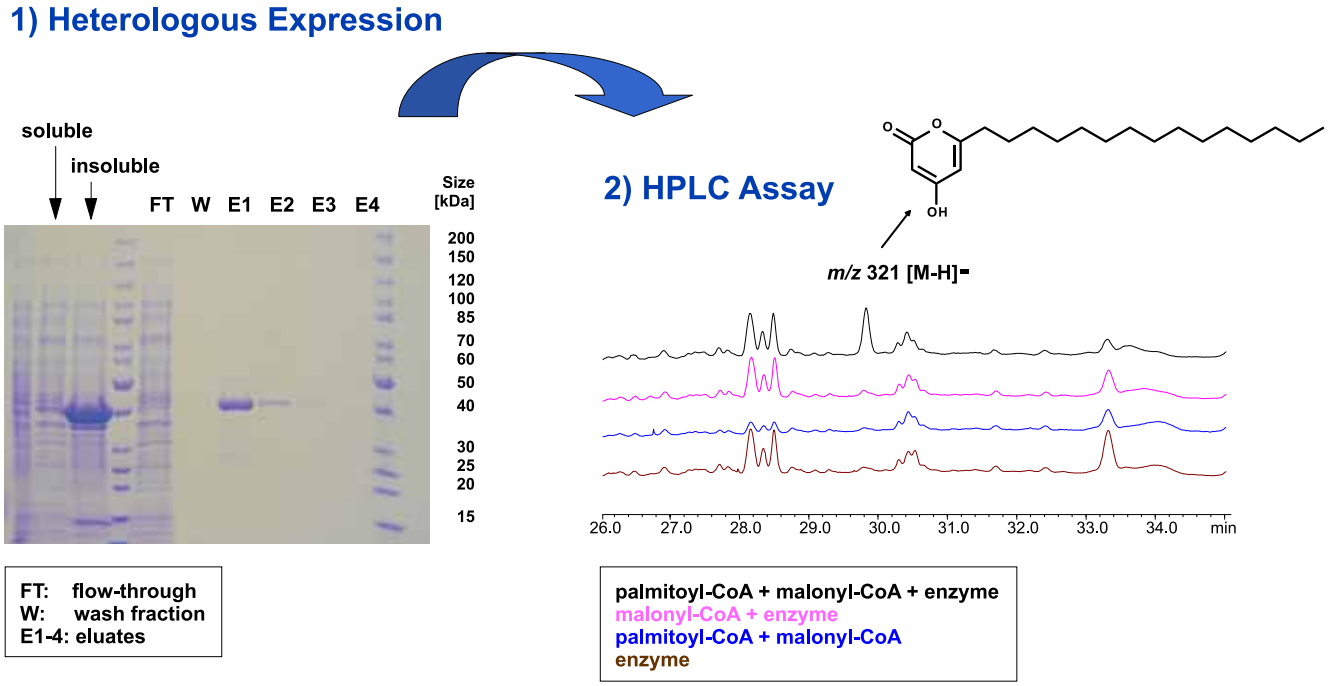

Figure 3
Purification of the histidine-tagged type-III PKS monitored via SDS-PAGE and preliminary functional characterization.

modimeric proteins that commonly employ acyl-coenzyme A thioesters as substrates and synthesize polyketides by successive condensation steps using a single catalytic center. It has previously been shown that protein sequence diversity within type-III PKSs correlates with functional differences. In order to assess the product that derives from the *Herpetosiphon* type-III PKS, a comprehensive phylogenetic analysis was performed, which placed the protein in a clade of sequences that are associated with the production of alkylresorcinols and alkylpyrones, respectively. This result was, however, inconsistent with genomic data, which suggested a role in the biosynthesis of 3,5-dihydroxyphenylglycine. In order to differentiate between both scenarios, the type-III PKS gene was expressed in *E. coli* as a hexahistidyl-tagged protein. An initial in vivo analysis of the recombinant strain did not reveal any significant metabolic differences when compared to a control strain harbouring an empty vector. Due to the low cellular pool of acyl-CoA thioesters in *E. coli*, this result was not too surprising and we decided to go on with the *in vitro* testing. Using palmitoyl-CoA and malonyl-CoA as substrates, we set up an HPLC based assay (Figure 3). The chromatograms obtained from three independent experiments clearly showed the presence of an additional peak in enzymatic reactions that contained both substrates. On the other hand, no conversion was observed when only malonyl-CoA was incubated with the protein. By means of mass spectrometry the new peak was identified as 4-hydroxy-6-pentadecyl-2-pyrone. It was thus evident that the type-III PKS gene encodes a pyrone synthase. Further functional characterization revealed that the type-III PKS possesses rather relaxed substrate specificity. The enzyme accepted both saturated and unsaturated, medium- and long-chain fatty acid CoA thioesters as well as aromatic acyl-CoA analogues as starter substrates, producing the corresponding triketide pyrones. RT-PCR analysis indicated that the type-III PKS gene

is constitutively expressed in *H. aurantiacus*. We now aim at identifying the biological function of alkylpyrones by means of gene inactivation studies.

3 Natural product discovery in microfluidic devices

Natural product competent microorganisms are rarely restricted to the biosynthesis of one single compound. They rather have the capability to synthesize a variety of metabolites in response to environmental changes. This well-known observation, which could be confirmed in the last decade by several sequencing projects, formed the basis behind the OSMAC (= One Strain – Many Compounds) approach. By variation of cultivation parameters, such as media composition, temperature, aeration, etc., it is possible to mimic environmental crosstalk and to enforce changes in the global regulation of metabolic pathways. In many cases, these changes exert profound effects on the transcriptome, proteome and ultimately metabolome of the organism. Though undirected, the OSMAC strategy has proven as an efficient and widely applicable method to trigger the expression of silent gene loci.

In collaboration with research groups at the Institute of Photonic Technology and at the HKI Bio Pilot Plant we are now planning to advance the OSMAC approach exploiting recent progress in the development of microfluidic devices. By scaling down the fermentation volume to few nanoliters on a chip-based platform, it will be possible to screen hundreds of different media for the presence of as yet untapped chemistry. In the first step, discrete microcultures of select antibiotic producing bacteria are generated via a two-phase segmented flow in a customized lab-on-a-chip (LOC) device. After a short incubation phase, the cultures are merged with nutrient medium droplets that have previously been inoculated with recombinant fluorescent test organisms. The latter are used to identify metabolic changes, as the low fermentation volume opposes chemical screening approaches. The growth of the test organism can be easily monitored in every compartment of the microchip by automated detection using a microscope with high-speed camera. Growth inhibitory effects that may indicate the release of antibiotic compounds are verified and analysed in our group. In summary, the LOC approach offers the advantages of a high throughput process, which makes it highly attractive for industrial applications. Culture conditions can be easily optimised towards induction of antibiotic production and improvement of expression levels. The lessons learned from this project may be of significant consequence in terms of charting a more effective course to utilize the idle genomic potential of microorganisms.

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DiNaMid: Genom-basierte Findung neuer antimikrobieller Naturstoffe in mikrofluidischen Chips; Identifizierung neuer Stoffwechselwege und Naturstoff-Isolierung
Markus Nett, Martin Roth

Selected publications

(HKI authors in bold)

Schneider P, Jacobs JM, Neres J, Aldrich CC, Allen C, **Nett M**, **Hoffmeister D** (2009) The global virulence regulators VsrAD and PhcA control secondary metabolism in the plant pathogen *Ralstonia solanacearum*. *ChemBioChem* 10, 2730-2732.

Nett M, Gulder TAM, Kale AJ, Hughes CC, Moore BS (2009) Function-oriented biosynthesis of β-lactone proteasome inhibitors in *Salinispora tropica*. *J Med Chem* 52, 6163-6167.

Penn K, Jenkins C, **Nett M**, Udwary DW, Gontang EA, McGlinchey RP, Foster B, Lapidus A, Podell S, Allen EE, Moore BS, Jensen PR (2009) Genomic islands link secondary metabolism to functional adaptation in marine Actinobacteria. *ISME J* 3, 1193-1203.

McGlinchey RP, **Nett M**, Eustaquio AS, Asolkar RN, Fenical W, Moore BS (2008) Engineered biosynthesis of antiprotealide and other unnatural salinosporamide proteasome inhibitors. *J Am Chem Soc* 130, 7822-7823.

McGlinchey RP, **Nett M**, Moore BS (2008) Unraveling the biosynthesis of the sporolide cyclohexenone building block. *J Am Chem Soc* 130, 2406-2407.

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Bio Pilot Plant

Bio Pilot Plant

The research of the HKI Bio Pilot Plant is mainly focused on the development, optimization and scale-up of biotechnological processes from flask cultures via laboratory scale to pilot scale. In addition technologies for the cultivation of microorganisms in a nano-litre scale were developed, which are to be used especially for the discovery of new antibiotics in slowly growing microorganisms.

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

INTRODUCTION | EINLEITUNG

Head:
Dr. Uwe Horn

Forschungsschwerpunkte des Biotechnikums sind die Entwicklung und Optimierung biotechnologischer Prozesse von Naturstoff-Produzenten. Das reicht von der Petrischale über den Labormaßstab bis zum Pilotmaßstab. Dazu stehen Fermenter bis zu 3.000l Nettovolumen sowie entsprechend großvolumige Aufarbeitungsmöglichkeiten zur Verfügung. Außerdem wurden Technologien für die Kultivierung von Mikroorganismen im Nanolitermaßstab entwickelt, die insbesondere für die Entdeckung von neuen Naturstoffen in langsam wachsenden Mikroorganismen eingesetzt werden sollen.

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

Um die Anforderungen der produzierenden Mikroorganismen zu analysieren und bei der Fermentation zu steuern, werden experimentelle und theoretische Methoden kombiniert.

Die intensive Zusammenarbeit des Biotechnikum mit den Forschungsabteilungen des HKI sowie Gruppen der Friedrich-Schiller-Universität Jena und der FH Jena kommt insbesondere in der zunehmenden Zahl gemeinsam betreuter Doktorarbeiten zum Ausdruck.

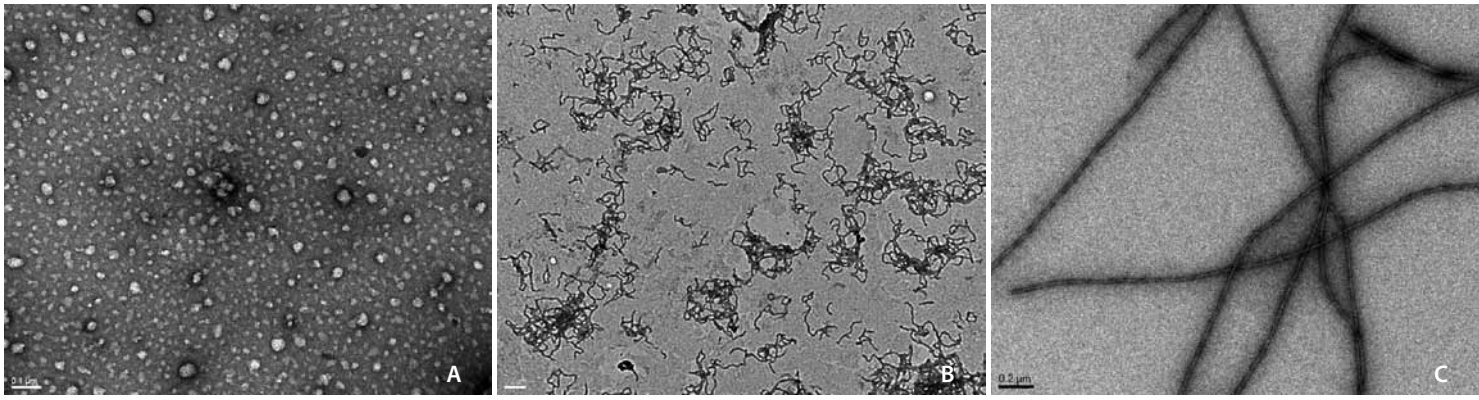

Figure 1
Electron microscope images of Aβ(1-40) (A) Oligomers, (B) Protofibrils, (C) mature fibrils

1 Directed selection and characterization of camelid VHH-domains directed to amyloid fibrils

Group Members: Karin Wieligmann, Magdalena Bereza, Uwe Horn

Recombinant libraries represent a modern and cost-effective source for the generation of monoclonal antibodies. The library constructed in our group is a fully synthetic library of camelid VHH-domains. Camelid antibodies significantly differ from conventional antibodies, because they are devoid of the light chain. The C-terminal VHH domain of this antibody is the smallest possible binding fragment. All antigen specificity is encoded within a single polypeptide sequence. Despite their small size and the presence of a single intramolecular disulfide bond, they are suited for functional expression in the periplasma of *E. coli*. All clones of our in-house library (~10^8) were preselected for functionality, solubility and correct folding.

Recombinant antibody fragments combine high stabilities with extremely high affinities and allow genetic manipulation and construction of transgenic cell lines. The recombinant availability of the selected VHH-domains enable genetic fusion to the alkaline phosphatase (AP). The AP-domain induces dimerisation of the whole VHH-AP construct. This leads to enhanced binding affinity and allows easy detection in immunoassays.

Our library can be read out by phage display in competetive selection environment. Using this approach we selected conformational specific antibodies directed against β-amyloid, a protein associated with Alzheimer's disease. There exist various conformational species of Aβ: intrisically disordered peptides, oligomers, protofibrils and fibrils – main constituents of extracelluraly deposited plaques in the brain. It is still under debate which of this species are most pathologically relevant but all are targeted therapeutically (Figure 1).

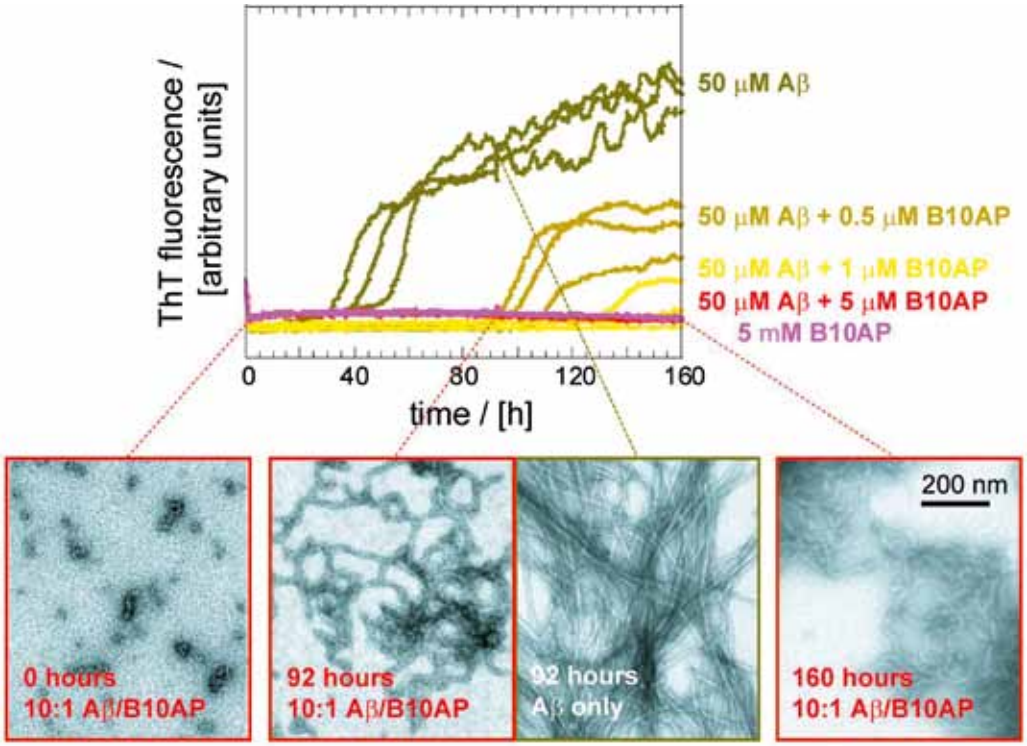

Figure 2
Aβ (1-40) fibril formation in presence and absence of B10AP monitored online with ThioflavinT (37°C) and transmission electron microscopy (TEM). (Habicht *et al.*, 2007)

We have generated antibody domains, termed B10, KW2 and KW3, which bind to Aβ amyloid fibrils, but neither to disaggregated (intrinsically disordered) Aβ peptide nor to Aβ oligomers (structural precursor of amyloid fibrils). This was assessed by surface plasmon resonance and immunoblot experiments.

In addition we tested their ability for binding to amyloid fibrils, generated from different polypeptide chains. Immunoblots showed that B10, KW2 and KW3 recognize amyloid fibrils from h-SAA, m-SAA, insulin, G-helix and glucagons. This reveals that the selected VHH-domains are conformation but not sequence specific binders.

Binding affinities were assessed with surface plasmon resonance. As a result we obtained dissociation constants of 7 nM for B10AP, 30 nM for KW2-AP and 11 nM for KW3-AP, respectively. Further examination of B10 proved that it not only binds to Aβ fibrils but also inhibits aggregation by stabilising the protofibril stage (Figure 2).

To determine which residues in the CDR regions are responsible for B10/Aβ fibrils recognition we replaced every amino acid in CDRs by alanine. It results in 24 B10 mutants, which were assessed for binding to Aβ fibrils with surface plasmon resonance. Most striking influence on binding to Aβ fibrils had two alanine mutans Arg39/Ala and Arg61/Ala but all positively charged residues replaced with alanine decreased binding affinity. This shows that ionic interactions are involved in B10 fibril recognition.

The project was carried out in collaboration with Peter Hortschansky; Dept. Molecular and Applied Microbiology and the Junior Research Group of Marcus Fändrich, MPG Halle.

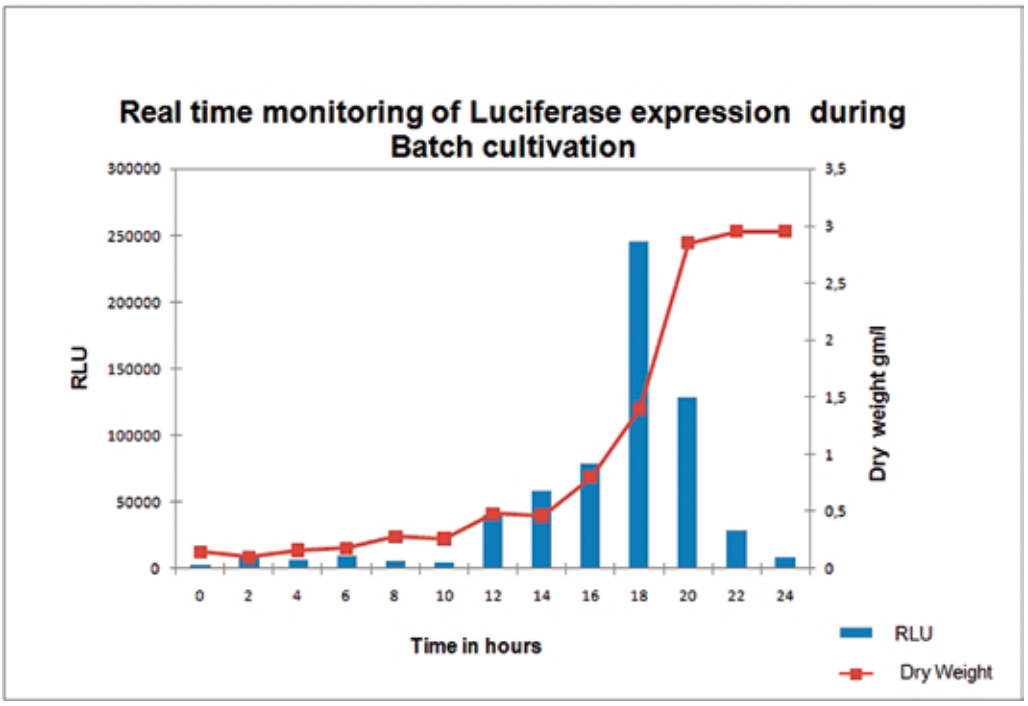

Figure 3
Activity of a secondary metabolite gene of *Aspergillus nidulans* during batch cultivation measured with the *Gaussia* luciferase system.

2 Regulation of secondary metabolite gene clusters in *Aspergillus nidulans*

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

The availability of fully sequenced fungal genomes has opened the doors to a wealth of information on putative secondary metabolite (natural product) gene clusters. Genome analyses show that surprisingly high numbers of polyketide synthase (PKS) gene clusters exist in filamentous fungi. Up to 28 PKS gene clusters have been speculated in the genome of the well-known model organism *Aspergillus nidulans* based on *in silico* analyses. Although recently a few metabolites could be identified, the products of a large number of PKSs remain unknown and little is known about their biological function and regulation.

Our goal is to activate these "silent" gene clusters and identify the corresponding products. By analysing regulatory elements and mechanisms we want to get to know more about common activation mechanisms of secondary metabolite clusters. Weak promotor activity and short time of expression make the gene regulation studies challenging.

Reporter system for measuring gene activity

We established a reporter system based on the marine copepod *Gaussia princeps* luciferase successfully in *A. nidulans* for measuring single gene activity. *Gaussia* luciferase is a marine luciferase which in contrast to other luciferases e.g. from *Photinus pyralis*, is ATP-independent and exhibits a flash kinetics. This means that a high intensity signal is generated and then decays over time thus resulting in a sensitive reporter assay. The luciferase also contains a eukaryotic secretion signal facilitating extracellular signal detection during various cultivation and screening techniques.

In cooperation
with the Departments of Molecular and Applied Microbiology (MAM) and Biomolecular Chemistry (BMC)

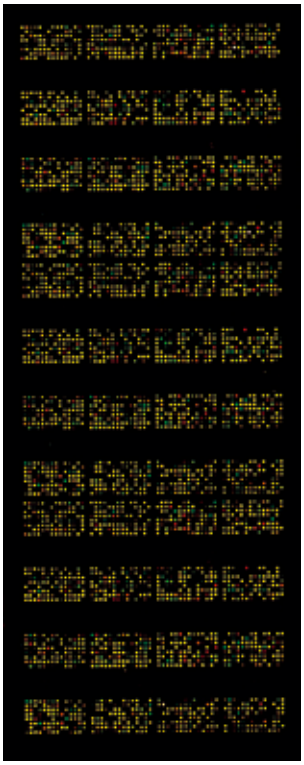

Figure 4
DNA Microarray chip for analysis of gene expression profiles.

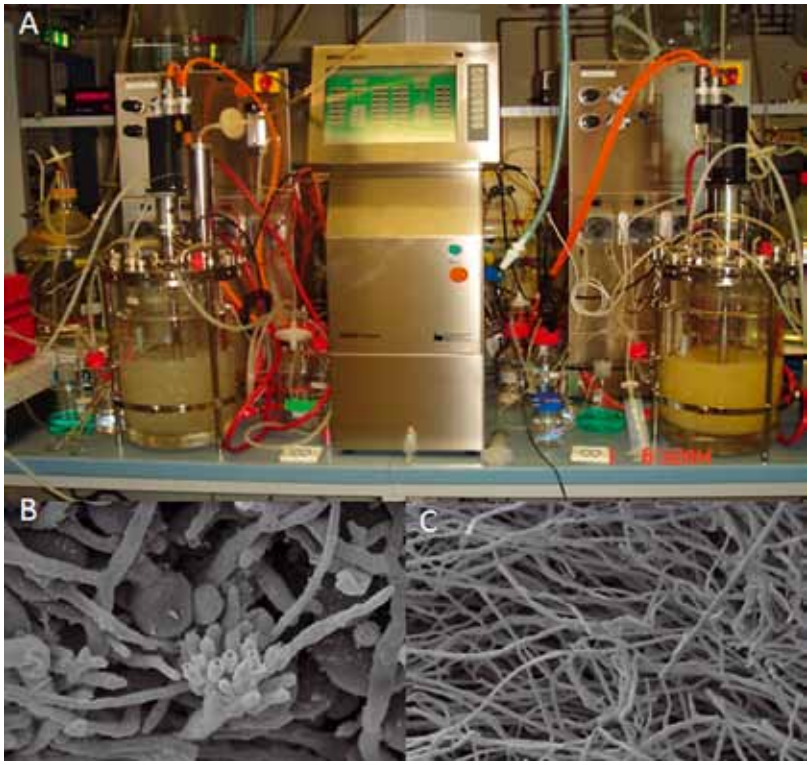

Figure 5
(A) depicts the BIOSTAT B-DCU Twin 5 Liter bench top fermentor used in continuous cultivation of the fungi. (B) Scanning electon micrographs of *Aspergillus nidulans* ascospore formed during submerged cultivation. (C) Scanning electron micrographs of the vegetative hyphae of *Aspergillus nidulans*.

Our results also show, that the half-life of this luciferase in *A. nidulans* cultivations is in the range of hours, enabling us to measure the kinetics of gene activations, in contrast to other reporter systems e.g. GFP-fusions (Figure 3).

The regulation mechanisms of genes can generally be divided into "global" (genome-wide) and "local" (cluster- or gene-specific) mechanisms. A prominent example for genome-wide regulation is the histone-acyltransferase LaeA involved in the regulation of the terrequinone-cluster (Shwab *et al.*, 2007). To the present knowledge, it catalyses the change of the DNA from inactive heterochromatin state to the active euchromatin state. Another global regulation mechanism is the methylation of DNA, where methylated DNA is inactive.

In our experiments we checked different deacetylase and methyltransferase inhibitors for their ability to activate certain secondary metabolite genes.

Local regulation mechanisms include transcription factors that specifically activate or inactivate cluster genes. Examples are the cluster-specific transcription factors leading to the activation of the aspyridone (Bergmann *et al.*, 2007) and asperfuranone cluster (Chiang *et al.*, 2009) in *A. nidulans*.

In combination with gene knock-out and overexpression strategies as well as protein-DNA interaction studies (surface plasmon resonance) we try to elucidate transcription factor based pathways involved in secondary metabolite regulation.

Continuous cultivation in combination with DNA microarray analyses

Continuous cultivation offers the possibility to grow microorganisms at a specific growth rate under defined nutrient limitation. This fermentation regime overcomes the influence of unpredictable growth and limitations arising in conventional flask cultivations. The

combination of continuous cultivation, genomic and metabolomic methods enables a global overview of the influence of specific parameters on gene activity or secondary metabolite formation.

We have established a continuous cultivation scheme for *Aspergillus nidulans*. The recombinant fungi were cultivated under various nutrient limitation regimes *viz* C, N, P limitations and at growth rates as high as 0.2/hour and as low as 0.05/hour. With the aid of the reporter assay we could mark the time window of the secondary metabolite gene expression. The differential gene expression arising due to specific nutrient limitation was investigated with DNA microarray (Figures 4 and 5).

Microbial crosstalk

Aspergillus nidulans is a saprophytic fungus. Mostly dwelling in the soil, where there is a fierce competition posed by competing niche dwellers like *Streptomyces sps*. One definition of secondary metabolite is that these molecules form a defense arsenal to defend their producer in a habitat where nutrient supply is sparse.

Recently, Schroeckh *et al.* (PNAS, 2009) have proven that the secondary metabolite profile of *Aspergillus nidulans* is affected by the physical proximity of *Streptomyces*. Our research goal is to probe into the volatile compounds generated by the competitor organism and check how these influence the metabolome of the organism of our interest.

Towards this end, we have designed:

1. Screening assays to screen for potential candidates.
2. Adopted 'closed loop stripping' model to the fermentor system.

3 Determination of the most common actinomycetes in water-damaged buildings

Group Leaders: Karin Martin, Ingrid Groth

Adverse health effects on people living in water-damaged buildings are mainly considered to be caused by spore forming fungi. However, it was shown by epidemiological studies that fungal colonization of humid environments is very often accompanied by bacteria, among them numerous isolates belonging to genera of the order Actinomycetales. In some of the studied samples members of this order were dominating. It is known that actinomycetes are able to produce a variety of secondary metabolites of high structural diversity. Actinomycetes, especially *Streptomyces* species, can provoke cytotoxic effects. Therefore a health risk for inhabitants of buildings contaminated by microbes can not be excluded.

The aim of the joint project headed by the Regierungspräsidium-Stuttgart – Landesgesundheitsamt (LGA) was

- to investigate the occurrence of actinomycetes in water-damaged buildings and detect "typical" actinomycetes involved in such processes,
- to provide praxis relevant and robust methods for isolation and rapid classification and identification of actinomycetes in contaminated indoor environments as a prerequisite for further taxonomic and infection biological studies, to define suitable media for the growth of a broad range of different actinomycetes, and
- to study cytotoxic effects of the contaminaited building materials and the actinomycete strains isolated from this material.

Isolation and taxonomic characterization of actinomycetes

On a workshop in Jena organized by the HKI, living cultures of relevant actinomycete reference strains from the HKI culture collection were used to demonstrate the morphological diversity of these microorganisms and the influence of culture conditions on growth and expression of morphological properties. Subsequent standard procedures for the isolation of actinomycetes were defined and four indoor samples were studied by the project partners in parallel to compare the applicability and reproducibility of the used methods. The isolates obtained by the partners were classified in taxonomic groups. Identical isolates were eliminated. For identification of the isolates at

Project partners:
Regierungspräsidium-Stuttgart – Landesgesundheitsamt (LGA)

Justus-Liebig-Universität, Institut für Angewandte Mikrobiologie, Gießen

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

Umweltmykologie Dr. Dill und Dr. Trautmann GbR, Berlin

Dr. Lorenz – Institut für Innenraumdiagnostik, Düsseldorf

Institut für Hygiene und Umweltmedizin – Klinikum RWTH Aachen

Figure 6
Isolates of the genera *Amycolatopsis*, *Nocardia*, *Nocardioides*, *Nocardiopsis*, *Promicromonospora* and *Pseudonocardia* on different media: ISP 3-agar (ISP3), Gauze-agar (Gauze), tryptone soy-agar (TSA), actinomycete-agar (Actino) and casein-mineral salt-agar (CM).

the genus or species level, the 16S rRNA gene sequences of representatives of each group were determined by the partner in Giessen.

A second cultivation-independent method was used to determine the occurrence of actinomycetes not culturable by the applied isolation method. The partner in Giessen extracted genomic DNA directly from samples of the contaminated building. 16S rRNA gene sequences were amplified by actinomycete-specific primers and subsequently cloned in *E. coli*. The clone library was analyzed and the next relatives defined by BLAST alignment.

Overall, 16 well characterised samples from water-damaged buildings were analysed.

Main results of the project

The results of the study show that the different materials from water-damaged buildings contain different and diverse actinomycete communities in high concentrations (range 1,8 x 10^4 - 7,6 x 10^7 CFU/g).

283 different actinomycete strains belonging to 35 genera of the order *Actinomycetales* were isolated. The most commonly detected genera were *Streptomyces*, *Amycolatopsis*, *Nocardiopsis*, *Nocardia*, *Pseudonocardia*, *Saccharopolyspora* and *Promicromonospora*. *Streptomyces* isolates were most abundant. Because actinomycete genera were found in most samples, it can be assumed that they are typical for humidity-damaged materials in indoor environments.

A significant correlation between the genus/species composition and the type of damp-building material was not detected, although such a differentiation in regard to nutrient composition, humidity, pH-value, micro- and surface structures of the materials in addition to the air humidity and

the temperature was reported in literature. The number of samples studied is too small to allow for general and statistically significant conclusions with regard to the different materials.

Because actinomycetes are highly diverse in their growth requirements it is not possible to isolate all representatives on one isolation medium. In this study, casein-mineral agar (CMA), brain-heart-infusion agar (BHI), CASO-agar and mineral agar according to Gauze were found to be suitable to culture a broad range of different actinomycetes. The appearance of different colony types and the morphological characteristics of actinomycetes may be quite different on the different agar media (Figure 6). For the detailed study of actinomycetes, which are able to form aerial mycelia and spores, ISP2 and ISP3 media are suitable, whereas actinomycetes not forming aerial mycelia should be studied on BHI and M79 media.

The analyses of the 16S rRNA gene sequences of the isolates in the course of the cultivation-based approach allowed for a more rapid and precise allocation at the genus or group level, than a morphological and chemotaxonomic characterisation. However, for species identification a morphological, biochemical and chemotaxonomic characterisation are necessary.

A higher diversity of actinomycetes was found with molecular cultivation-independent methods compared to culture based methods. In the 16 analysed material samples 59 genera of *Actinobacteria* were detected: 27 by molecular methods, 10 by the cultivation approach, and 22 by applying both methods. For this reason the application of both, cultivation-dependent and cultivation-independent methods for the study of the diversity of actinomycetes in humidity-damaged materials is highly recommended.

Description of new actinomycete taxa

The isolates obtained by each partner were classified in taxonomic groups according to their morphology and the presence of the isomers of the diaminopimelic acid in the cell wall peptidoglycan. The following 16S rRNA gene sequence comparison allowed an identification of the isolates at the genus or species level. Some isolates formed separated phylogenetic branches in the according phylogenetic tree of their relatives. By a polyphasic taxonomic approach using morphological, chemotaxonomical and molecular biological methods six of these isolates were recognized and described as new species of actinomycete genera: *Kytococcus aerolatus* sp. nov., *Citricoccus parietis* sp. nov., *Prauserella muralis* sp. nov., *Promicromonospora umidemergens* sp. nov., *Microlunatus parietis* sp. nov., and *Jiangella muralis* sp. nov., isolated from indoor environments. (Figures 7–9)

Figure 7
5-Be-005T *Prauserella muralis* sp. nov.

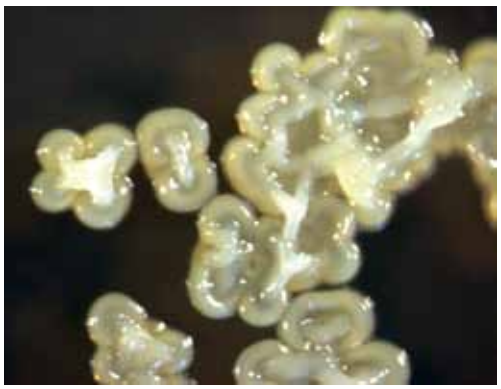

Figure 8
09-Be-007T *Promicromonospora umidemergens* sp. nov.

Figure 9
15-Je-017T *Jiangella muralis* sp. nov.

The results of the project are published in detail:
Schäfer J, Trautmann C, Dill I, Fischer G, Gabrio T, Groth I, Jäckel U, Lorenz W, Martin K, Miljanic T, Szewzyk R, Weidner U, Kämpfer P (2009). Vorkommen von Actinomyceten in Innenräumen. Springer-VDI-Verlag GmbH&Co. KG, Gefahrstoffe-Reinhaltung der Luft 9/2009, 335-341.

Because of high discriminative features the strains 14-Be-013^T and 02-Gi-014 were not affiliated to a known genus. Comparative analysis of 16S rRNA gene sequences showed that these bacteria are most closely related to genera within the family *Nocardiopsaceae*, but form a separate lineage within this family. The strains showed highest sequence similarities to *Marinactinospora thermotolerans*, *Nocardiopsis dassonvillei* susp. *albirubida* and *Nocardiopsis lucentensis*. Phenotypic analysis further differentiated strains 14-Be-013^T and 02-Gi-014 from the most closely related *Marinactinospora* and *Nocardiopsis* genera. Therefore the name *Murinocardiopsis flavida* gen. nov., sp. nov. was proposed for this novel taxon with the type species 14-Be-013^T. (Figures 10 and 11)

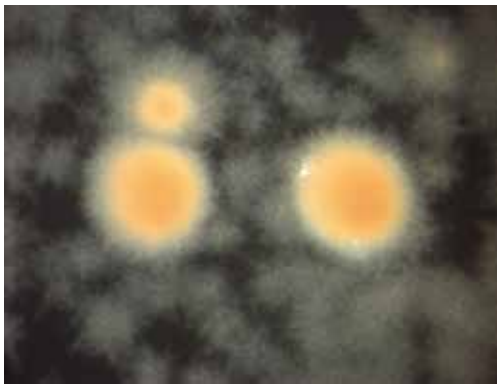

Figure 10
Murinocardiopsis flavida gen. nov., sp. nov. Type strain 14-Be-013^{T}on ISP3 Medium

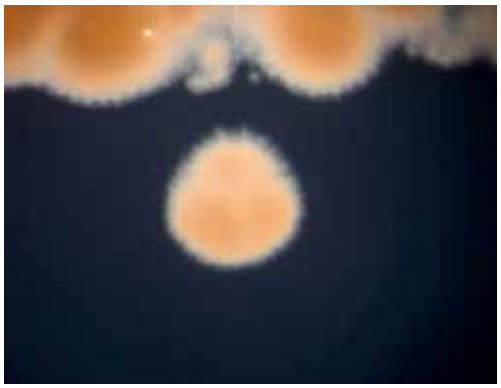

Figure 11
Murinocardiopsis flavida gen. nov., sp. nov. 02-Gi-014 on M79

5 Production of microbial biomass and natural products within the Internal Product Line, for research projects of HKI groups and external academic partners

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

A large number of microbial products from different microorganisms were produced within the scope of the Internal Product Line (IPL) and for partners from academia. 430 fermentations at the 1 to 300 liter scale were performed within 2008-2009 including preparation of inoculum cultures, fermentation and monitoring of the fermentations by examination of samples and data acquisition. Downstream processing has been performed: Separation of supernatant and biomass, fractionation of supernatant by preparative HPLC or adsorption on synthetic resins, solvent extraction, and concentration and freeze drying of the extracts. In 2008-2009 products from about 8,000 liter fermentation broth were prepared.

Collaborations within the IPL of HKI and with external academic partners

Project:
Investigation of secondary metabolite biosyntheses in *Burkholderia* species (HKI, Dept. Biomolecular Chemistry)
Activities at Bio Pilot Plant:
Pilot scale fermentation of *Burkholderia rhizoxinica* and *B. thailandensis* strains and downstream processing to isolate secondary metabolites.

Project:
Genome mining in clostridia (HKI, Dept. Biomolecular Chemistry)
Activities at Bio Pilot Plant:
Anaerobic cultivation of *Clostridium cellulolyticum* at 1 to 30 liter scale for isolation of novel natural products.

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

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

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

Project:
Secondary metabolism of predatory bacteria (HKI, Junior Research Group)
Activities at Bio Pilot Plant:
Pilot scale fermentation of predatory bacteria and downstream processing to isolate new natural products .

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

Project:
Potential use of heavy metal resistant streptomycetes in bioremediation (University Jena, Institute of Microbiology, Microbial Phytopathology)
Activities at Bio 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.

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External funding

Deutsche Forschungsgemeinschaft
Excellence Graduate School Jena School for Microbial Communication JSMC
Teilprojekt: Development of a procedure to avoid misincooperation of rare amino acids into therapeutic proteins during High Cell Density Fermentation
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Bundesministerium für Bildung und Forschung
DiNaMid: Genom-basierte Findung neuer antimikrobieller Naturstoffe in mikrofluidischen Chips; Identifizierung neuer Stoffwechselwege und Naturstoff-Isolierung
Martin Roth, Markus Nett

Bundesministerium für Bildung und Forschung
SERIZELL: Hochdurchsatz-Bioassay-System auf Basis mikroserieller Zellkulturen in flüssig/flüssig-Zweiphasensystemen
Teilprojekt: Systemevaluierung mit ausgewählten Bioassays
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Entwicklung eines rekombinanten Wirt-Vektor-Systems für die Biosynthese von 10-deacetylbaccatin III-10-O-Acetyltransferase
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Synthetische und biotechnologische Herstellung eines Mykotoxins
Teilprojekt: Biotechnologische Herstellung
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Kameliden Antikörper basierter ELISA zur simultanen Bestimmung relevanter Mykotoxine
Uwe Horn, Hans Krügel

Selected publications
(HKI authors in bold)

Huang H, He J, Niu X, **Menzel K-D**, **Dahse HM**, **Grabley S**, Fiedler P, **Sattler I**, **Hertweck C** (2008) Benzopyrenomycin, a cytotoxic bacterial polyketide metabolite with an unprecedented benzo[a]pyrene-type carbacyclic ring system. *Angew Chem Int Ed* 47, 3995-3998.

Borowski A, Kuepper M, **Horn U**, **Knüpfer U**, Zissel G, Hähne K, Luttmann W, Krause S, Virchow jr J C, Friedrich K (2008) Interleukin-13 acts as an apoptotic effector on lung epithelial cells and induces pro-fibrotic gene expression in lung fibroblasts. *Clinical and Experimental Allergy* 38, 619-628.

Li Y-Q, Huang X-S, Ishida K, Maier A, Kelter G, Jiang Y, **Peschel G**, **Menzel K-D**, Li M-G, Wen ML, Xu L-H, Grabley S, Fiebig H-H, Jiang C l, Hertweck C, Sattler I (2008) Plasticity in Gilvocarcin-Type C-Glycoside Pathway: Discovery and Antitumoral Evaluation of Polycarcin V from *Streptomyces polyformus*. *Org Biomol Chem* 6, 3601-3605.

Richter ME, Traitcheva N, **Knüpfer U**, Hertweck C (2008) Sequential asymmetric polyketide heterocyclization catalyzed by a single cytochrome P450 monooxygenase (AurH). *Angew Chem Int Ed Engl* 47, 8872-8875.

Schachtschabel D, David A, **Menzel K-D**, Schimek C, Wöstemeyer J, Boland W (2008) Cooperative biosynthesis of trisporoids by the (+) and (-) mating types of the zygomycete *Blakeslea trispora*. *ChemBioChem* 9, 2004-2008

Kawe M, **Horn U**, Plückthun A (2009) Facile promoter deletion in *Escherichia coli* in response to leaky expression of very robust and benign proteins from common expression vectors. *Microbial Cell Factories* 8, 8.

Schroeckh V, **Scherlach K**, **Nützmann H-J**, **Shelest E**, **Schmidt-Heck W**, **Schuemann J**, **Martin K**, **Hertweck C**, **Brakhage AA** (2009) Intimate bacterial–fungal interaction triggers biosynthesis of archetypal polyketides in *Aspergillus nidulans*. *Proc Natl Acad Sci USA* 106, 14558-14563.

Funfak A, Hartung R, Cao J, **Martin K**, Wiesmüller K-H, Wolfbeis OS, Köhler JM (2009) Highly resolved dose–response functions for drug-modulated bacteria cultivation obtained by fluorometric and photometric flow-through sensing in microsegmented flow. *Sens Actuators B* 142, 66-72.

Kensy F, **Zang E**, Faulhammer C, Tan RK, Büchs J (2009) Validation of a high-throughput fermentation system based on online monitoring of biomass and fluorescence in continuously shaken microtiter plates. *Microb Cell Fact* 8, 31.

Kusebauch B, Busch B, Scherlach K, **Roth M**, Hertweck C (2009) Polyketide chain branching by an enzymatic Michael Addition. *Angew Chem Int Ed* 48, 5001-5004.

Collaborations

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Universität Bonn
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Institut für Bioanalytik, Umwelttoxikologie und Biotechnologie GmbH, Halle

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Merck KGaA, Darmstadt

Sanofi-Aventis Deutschland GmbH

Tepha Inc., Lexington, USA

WERNER BioAgents, Jena

Internal Product Line

Internal Product Line

In order to identify 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. All these efforts are brought together by the Internal Product Line (IPL).

The IPL team directs and coordinates these all activities within the HKI and with external collaborators. It consists of a group of scientists representing the diverse expertise required for the discovery, identification and characterization of natural products. The IPL team establishes, maintains and improves the basic methods and platform technologies that are needed for a professional preclinical investigation of novel structures. This includes strain collection, compound libraries, assay methods and databases. In addition, the IPL team organizes screening hierarchies, substance flows and data management as well as the interdisciplinary analysis and biological profiling of promising hits. Longstanding contacts to industry partners and a close collaboration with the technology transfer agency Ascenion allow the successful marketing of existing and new property rights.

Some of the departments and research groups provide the IPL with microbial strains, extracts, natural products or synthetic derivatives. Others identify new targets or develop and run novel assay systems for the identification of novel lead structures. Elucidation and optimization of biosynthetic pathways,

INTRODUCTION | EINLEITUNG

Coordinator:
Dr. Michael Ramm

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.

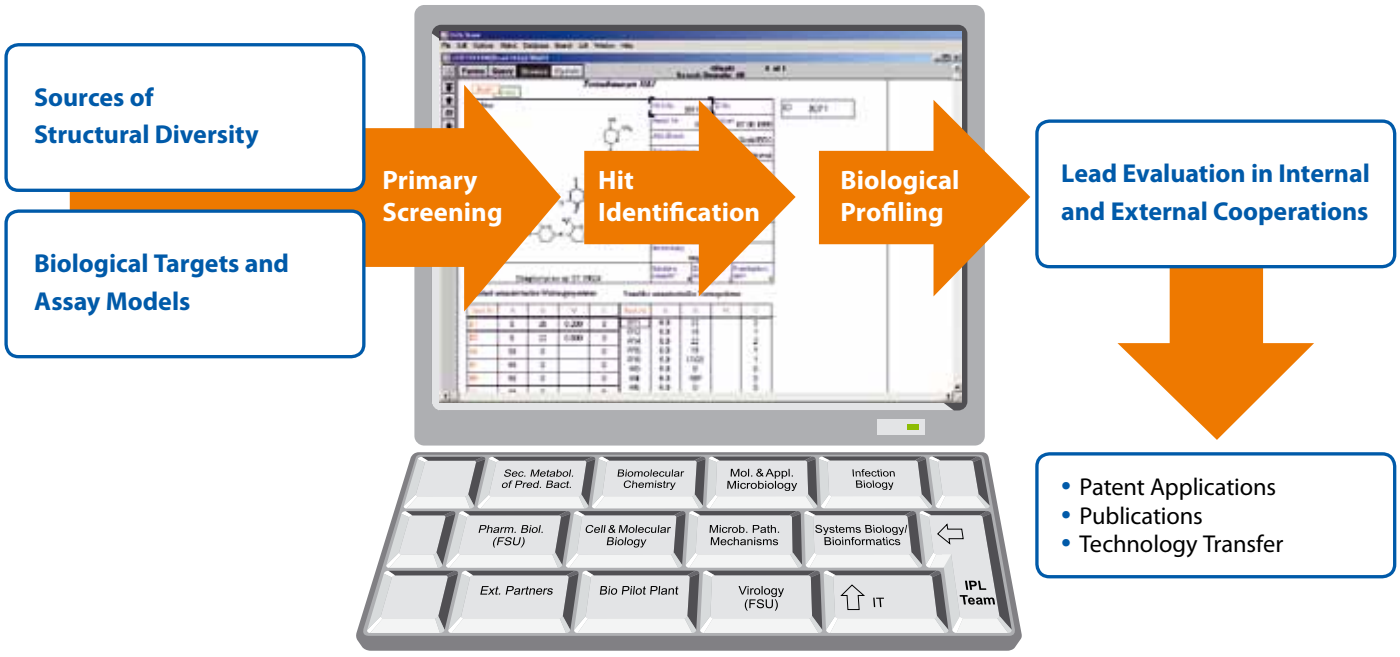
Alle damit verbundenen Aktivitäten innerhalb des HKI und mit externen Partnern werden vom DBL-Team koordiniert. Es setzt sich aus Fachleuten aller Abteilungen und Forschungsgruppen zusammen, die mit ihrer individuellen Expertise in alle Stufen der Bearbeitung pharmakologisch interessanter neuer Naturstoffe und Synthetika 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 Mikroorganismen-Stämme, Extrakte, Naturstoffe und/oder synthetische Derivate zur DBL bei. Andere Gruppen identifizieren neue Targets, entwickeln Testsysteme und nutzen diese für die

strain improvement, pilot scale fermentation and downstream processing are carried out for natural products of primary interest. New technologies, like transcription profile analysis, proteome analysis and metabolomics, complement the methods applied.
Identified lead compounds are characterized in their function as mediators of biological communication. The IPL team evaluates the natural products as tools for modern biotechnology or as therapeutic agents. The assays established for identification and characterization of natural products include a variety of biological communication systems. They enable studies of interactions between natural products and biological macromolecules and of their effects within biological networks. This includes molecular interactions of natural products with microorganisms but also with higher organisms including complex pathogen/host-interactions.
The IPL focuses on the identification and adaptation of new antifungal lead compounds which might be used against difficult to treat fungal pathogens in humans.

Suche nach neuen Leitstrukturen. Für Naturstoffe von vorrangigem Interesse werden Biosynthesewege untersucht, die Produktbildner optimiert, sie werden im Technikumsmaßstab kultiviert 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. 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	Michael Ramm
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Cell and Molecular Biology	Peter Gebhardt Frank Hänel
Infection Biology	Hans-Martin Dahse
Microbial Pathogenicity Mechanisms	Ilse Jacobsen
Molecular and Applied Microbiology	Christiane Weigel
Bio Pilot Plant	Karin Martin Martin Roth
Secondary Metabolism of Predatory Bacteria	Markus Nett
Systems Biology / Bioonformatics	Reinhard Guthke
Administration / IT-Group	Reinald Schorcht
University Jena, Institute of Pharmacy	Dirk Hoffmeister
Jena University Hospital, Institute of Virology and Antiviral Therapy	Michaela Schmidtke

HKI culture collection

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

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

HKI compound libraries

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

HKI assays

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

HKI database

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

Selected projects

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

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

Benzothiazinones – In our effort to discover new leads to counter the tuberculosis (TB) pandemic we synthesized and characterized 1,3-benzothiazin-4-ones (BTZs). BTZs are a new class of antimycobacterial agents that kill *Mycobacterium tuberculosis in vitro, ex vivo*, and in mouse models of TB. Within the EU-funded project "New medicines for tuberculosis" (NM4TB) the enzyme decaprenylphosphoryl-

Han L, Huang X, Dahse HM, Möllmann U, Grabley S, Lin W, Sattler I (2008) *Planta Med* 74, 432-437.

Huang H, He J, Niu X, Menzel K-D, Dahse HM, Grabley S, Fiedler P, Sattler I*, Hertweck C* (2008) *Angew Chem Int Ed* 47, 3995-3998. * equal contribution.

Mies KA, Gebhardt P, Möllmann U, Crumbliss AL (2008) *J Inorg Biochem* 102, 850-861.

Niu X, Dahse HM, Menzel K-D, Lozach O, Walther G, Meijer L, Grabley S, Sattler I (2008) *J Nat Prod* 71, 689-692.

Mahboobi S, Eichhorn E, Winkler M, Möllmann U (2008) *J Med Chem* 43, 633-656.

Makarov V, Manina G, Mikusova K, Möllmann U, Ryabova O, Saint-Joanis B, Dhar N, Pasca MR, Buroni S, Lucarelli AP, Milano A, De Rossi E, Belanova M, Bobovska A, Dianiskova P, Kordulakova J, Sala C, Fullam E, Schneider P, McKinney JD, Brodin P, Christophe T, Waddell S, Butcher P, Albrethsen J, Rosenkrands I, Brosch R, Nandi V, Bharath S, Gaonkar S, Shandil RK, Balasubramanian V, Balganesh T, Tyagi S, Grosset J, Riccardi G, Cole ST (2009) *Science* 324, 801-804.

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

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

Brock M, Ibrahim-Granet O. Bioluminescent strain *Aspergillus fumigatus* PCT/EP2009/062340

Abdou R,Scherlach K, Dahse HM, Sattler, Hertweck C. Botryosphaerones, novel depsidones and their use as medicaments. EP 09 003 841.5

Schroeckh V, Scherlach K, Nützmann HW, Shelest E, Schmidt-Heck W, Schümann J, Martin K, Hertweck C, Brakhage AA (2009) *Proc Natl Acad Sci* USA 106, 14558-14563.

Herzigova P, Klimesova V, Palat K, Kaustova J, Dahse HM, Möllmann U (2009) *Arch Pharm Chem Life Sci* 342, 394-404.

Miethbauer S, Gaube F, Möllmann U, Dahse HM, Schmidtke M, Gareis M, Pickhardt M, Liebermann B (2009) *Planta Medica* 75, 1-3.

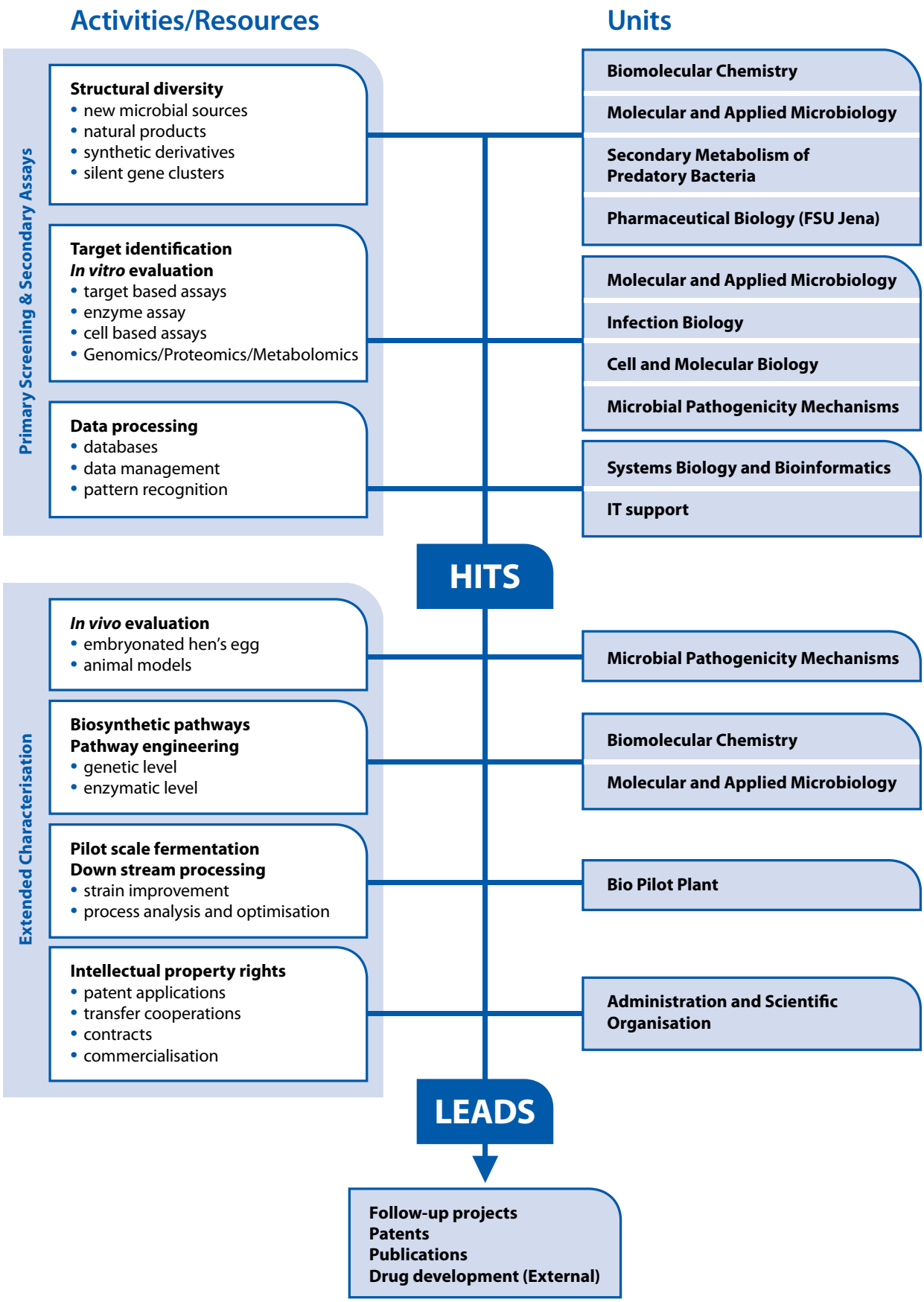
Miller MJ, Zhu H, Xu Y, Wu C, Walz AJ, Vergne A, Roosenberg JM, Moraski G, Minnick AA, McKee-Dolence J, Hu J, Fennel K, Dolence EK, Dong L, Franzblau S, Malouin F, Möllmann U (2009) *Biometals* 22, 61–75.

Möllmann U, Heinisch L, Bauernfeind A, Koehler T, Ankel-Fuchs D (2009) *Biometals* 22, 615–624.

Sammer UF, Völksch B, Möllmann U, Schmidtke M, Spiteller P, Spiteller M, Spiteller D (2009) *Appl Environ Microbiol* 75, 7710-7717.

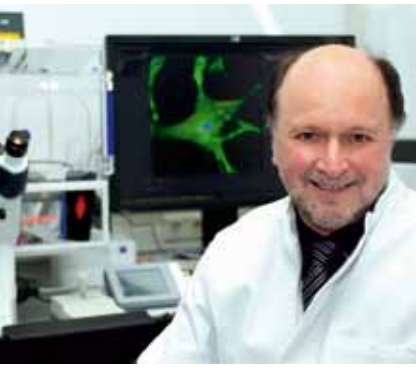
β-D-ribose 2'-epimerase was identified as a major BTZ target by cooperation partners. Inhibition of this enzymatic activity abolishes the formation of decaprenylphosphoryl arabinose, a key precursor that is required for the synthesis of the cell-wall arabinans, thus provoking cell lysis and bacterial death. BTZs were identified using a combination of activity-guided chemical and biotechnological techniques followed by extensive biological profiling within the IPL. The most advanced compound, BTZ043, is a candidate for inclusion in combination therapies for both drug-sensitive and extensively drug-resistant TB.

Internal Product Line (IPL)
Durchgehende Bearbeitungslinie (DBL)



International Leibniz Research School for Microbial and Biomolecular Interactions

International Leibniz Research School for Microbial and Biomolecular Interactions



Speaker:
Prof. Peter Zipfel

The "International Leibniz Research School for Microbial and Biomolecular Interactions Jena" (ILRS) is the first graduate school at an institute of the Leibniz Association. Initially financed by competitively granted means of the Joint Initiative for Research and Innovation, it is now an integral part of structured doctoral training in the life sciences in Jena. In addition to the Leibniz Institute for Natural Product Research and Infection Biology – Hans Knöll Institute (HKI), the Friedrich Schiller University Jena (FSU) and the Max Planck Institute for Chemical Ecology (MPICE) are member institutions of the ILRS.
With the ILRS, the Hans Knöll Institute significantly contributed to Jena's profile as a centre of microbiological research in Germany and Europe. Already existing research activities in the field of microbiology are brought together and focussed on the aspect of interaction and communication of microorganisms. Thus, an environment for excellent scientific performance is created. More than half of the PhD students of the graduate school come from abroad, thereby enhancing international networking and lastingly increasing Jena's international attractiveness.
Scientific topics of the ILRS cover interactions of microorganisms with each other as well as with higher organisms (e. g. as pathogens), natural products involved in these interactions as well as analysis and evaluation of the

Coordinator:
Dr. Dorit Schmidt

Die „International Leibniz Research School for Microbial and Biomolecular Interactions Jena" (ILRS) ist die erste Graduiertenschule an einem Institut der Leibniz-Gemeinschaft. Sie wurde zunächst aus kompetitiv vergebenen Mitteln des Paktes für Forschung und Innovation finanziert und ist heute ein fester Bestandteil der strukturierten Graduiertenförderung auf dem Gebiet der Lebenswissenschaften in Jena. Neben dem Leibniz-Institut für Naturstoff-Forschung und Infektionsbiologie – Hans-Knöll-Institut (HKI) – sind die Friedrich-Schiller-Universität Jena (FSU) und das Max-Planck-Institut für chemische Ökologie (MPICE) beteiligt.
Mit der ILRS hat das Hans-Knöll-Institut einen wesentlichen Beitrag zur Profilierung des Standortes Jena als Zentrum der mikrobiologischen Forschung in Deutschland und Europa geschaffen. Die in Jena vorhandenen Forschungsaktivitäten auf dem Gebiet der Mikrobiologie werden gebündelt und auf den Aspekt Wechselwirkung und Kommunikation von Mikroorganismen fokussiert. Hierdurch werden die Voraussetzungen für herausragende wissenschaftliche Leistungen geschaffen. Mehr als die Hälfte der Doktoranden der Graduiertenschule kommt aus dem Ausland. Dies fördert die internationale Vernetzung und erhöht nachhaltig die internationale Attraktivität Jenas.
Wissenschaftliche Themen der ILRS sind die Interaktionen von Mikroorganismen untereinander sowie mit höheren Organismen (z. B. als Pathogene), deren stoffliche Grundlage sowie die Analyse und Bewertung der experi-

experimentally gained data by using bioinformatic methods. Microorganisms do not occur in nature as single cells, but live in very different habitats in close association with other organisms. These associations range from mutual symbiosis to parasitic interactions. Basis for all such communication processes are substances that are synthesized by the organisms involved and that are mutually sensed. A better understanding of the so far unexplored paths of communication will therefore help to identify new biologically active substances which have been optimized in terms of their effects in the course of evolution.

In 2009 the first PhD students successfully defended their PhD theses. The groups of Prof. Maria Mittag (FSU Jena), PD Dr. Christine Skerka and Dr. Matthias Brock (both HKI) were accepted as new members of the ILRS faculty. Furthermore, the second generation of PhD students was also recruited during the year 2009. Two calls for application resulted in almost 700 applications from 38 nationalities. The first 13 of the newly recruited PhD students have already started their research projects, a further eight students will arrive in Jena in 2010.

On August 01, 2008, Prof. Axel Brakhage, initiator and speaker of ILRS since 2005, passed this office on to Prof. Peter Zipfel (HKI). Prof. Johannes Wöstemeyer (FSU) and Prof. Wilhelm Boland (MPICE) are deputy speakers of ILRS.

mentell gewonnenen Daten mit den Methoden der Bioinformatik. Mikroorganismen kommen in der Natur nicht als Einzelzellen vor, sondern leben in den verschiedensten Habitaten im Verbund mit anderen Organismen. Hierzu gehören mutualistische Symbiosen ebenso wie parasitäre Interaktionen. Grundlage sämtlicher Kommunikationsprozesse sind Substanzen, die von den beteiligten Organismen synthetisiert und wechselseitig wahrgenommen werden. Ein besseres Verständnis der bisher weitgehend unerforschten Kommunikationswege dient damit der Auffindung neuer biologisch aktiver Substanzen, die im Laufe der Evolution auf natürlichem Wege in ihrer Wirkung optimiert wurden.

Im Jahr 2009 haben die ersten Doktoranden ihre Dissertationen mit sehr großem Erfolg verteidigt. Die Gruppen von Prof. Maria Mittag (FSU Jena) sowie von PD Dr. Christine Skerka und Dr. Matthias Brock (beide HKI) wurden neu in die Graduiertenschule aufgenommen.

Ebenfalls im Jahr 2009 wurde die zweite Generation Doktoranden rekrutiert. In zwei Ausschreibungsrunden bewarben sich insgesamt knapp 700 Studenten aus 38 Nationen. Die ersten 13 Doktoranden haben ihre Forschungsarbeit bereits begonnen, weitere acht werden Anfang 2010 folgen.

Prof. Axel Brakhage, Initiator und Sprecher der ILRS seit 2005, gab diese Funktion am 1.8.2008 an Prof. Peter Zipfel (HKI) ab, die stellvertretenden Sprecher sind Prof. Johannes Wöstemeyer (FSU Jena) und Prof. Wilhelm Boland (MPICE).

In the framework of the structured PhD training the young scientists participate in method-specific internships and organize their own symposium and colloquium series, fostering scientific exchange with internationally renowned researchers in their field. In addition to the organization of the technical aspects of the training, the graduate school's coordinator is responsible for comprehensive socio-cultural care for the students, which helps in establishing a sense of community among the PhD students. Included in the performance catalogue of the ILRS Jena are also activities to spread research results to the general public. All training-relevant measures are completed by the PhD students according to a *Programme of Study* developed for the ILRS. This ensures highly efficient qualification of scientific junior staff within a very strict time frame.

Im Rahmen der strukturierten Doktorandenausbildung nehmen die jungen Wissenschaftler an methodenspezifischen Praktika teil, veranstalten eigene Symposien- und Kolloquienreihen und pflegen den wissenschaftlichen Kontakt zu international renommierten Forschern ihres Fachgebietes. Neben der Organisation dieser fachlichen Seite der Ausbildung ist die Koordinatorin der Graduiertenschule für eine umfassende soziokulturelle Betreuung der Doktoranden verantwortlich, die sich positiv auf die Gemeinschaftsbildung auswirkt. Zum Leistungskatalog der ILRS Jena gehört auch die öffentlichkeitswirksame Verbreitung der Forschungsergebnisse. Alle ausbildungsrelevanten Maßnahmen werden von den Doktoranden entsprechend einem für die ILRS entwickelten *Programme of Study* absolviert. Dies sichert eine hocheffiziente Qualifikation des wissenschaftlichen Nachwuchses in einem sehr stringenten Zeitschema.

PhD student	Supervisor
Interactions between microorganisms	
Swantje Behnken	Prof. Christian Hertweck
Anne Behrend	Prof. Wilhelm Boland
Susann Erdmann	Prof. Erika Kothe
Alexander Funk	Dr. Uwe Horn
Huijuan Guo	Prof. Wilhelm Boland
Christoph Heddergott	Prof. Johannes Wöstemeyer
Gerald Lackner	Prof. Christian Hertweck
Anita MacNelly	Prof. Gabriele Diekert
Anindita Sarkar	Dr. Uwe Horn
Sarbani Sarkar	Prof. Johannes Wöstemeyer
Dominik Senftleben	Prof. Erika Kothe
Lidan Ye	Prof. Gabriele Diekert

Institute	Project title
Leibniz Institute for Natural Product Research and Infection Biology – Hans Knöll Institute Department of Biomolecular Chemistry	Genome mining of Gram-positive bacteria for secondary metabolites
Max Planck Institute for chemical Ecology Department of Bioorganic Chemistry	Induction of metabolic shifts in Streptomyces
Friedrich Schiller University Jena Institute of Microbiology – Microbial Phytopathology	Expression and localization of G-protein coupled pheromone receptor Bar2 in the basidiomycete *Schizophyllum commune*
Leibniz Institute for Natural Product Research and Infection Biology – Hans Knöll Institute Bio Pilot Plant	Regulation of polyketide synthase gene clusters in *Aspergillus nidulans*
Max Planck Institute for chemical Ecology Department of Bioorganic Chemistry	Isolation, purification, and structural elucidation of active compounds from the tissue of insect
Friedrich Schiller University Jena Institute of Microbiology – General Microbiology and Microbial Genetics	Secretome analysis/ pathogenicity mechanisms of *Arthroderma benhamiae*
Leibniz Institute for Natural Product Research and Infection Biology – Hans Knöll Institute Department of Biomolecular Chemistry	The genome of the endofungal bacterium *Burkholderia rhizoxinca*
Friedrich Schiller University Jena Institute of Microbiology – Applied and Environmental Microbiology	Expression control and biosynthesis of dehalogenating enzymes from anaerobic soil bacteria in response to the interaction with aerobic halogenating fungi
Leibniz Institute for Natural Product Research and Infection Biology – Hans Knöll Institute Bio Pilot Plant	Induction of *Aspergillus nidulans* polyketide synthase genes by modulating cultivation parameters in continuously stirred tank bioreactor
Friedrich Schiller University Jena Institute of Microbiology – General Microbiology and Microbial Genetics	Sexual and parasitic interactions in *Mucor*-like fungi: Regulation of genes for the communication molecule trisporic acid
Friedrich Schiller University Jena Institute of Microbiology – Microbial Phytopathology	A surface hydrophobin in ectomycorrhiza interaction
Friedrich Schiller University Jena Institute of Microbiology – Applied and Environmental Microbiology	Studies on the microbial halogen cycle: reactions of fungal peroxidases and bacterial reductive dehalogenases

Continuation →

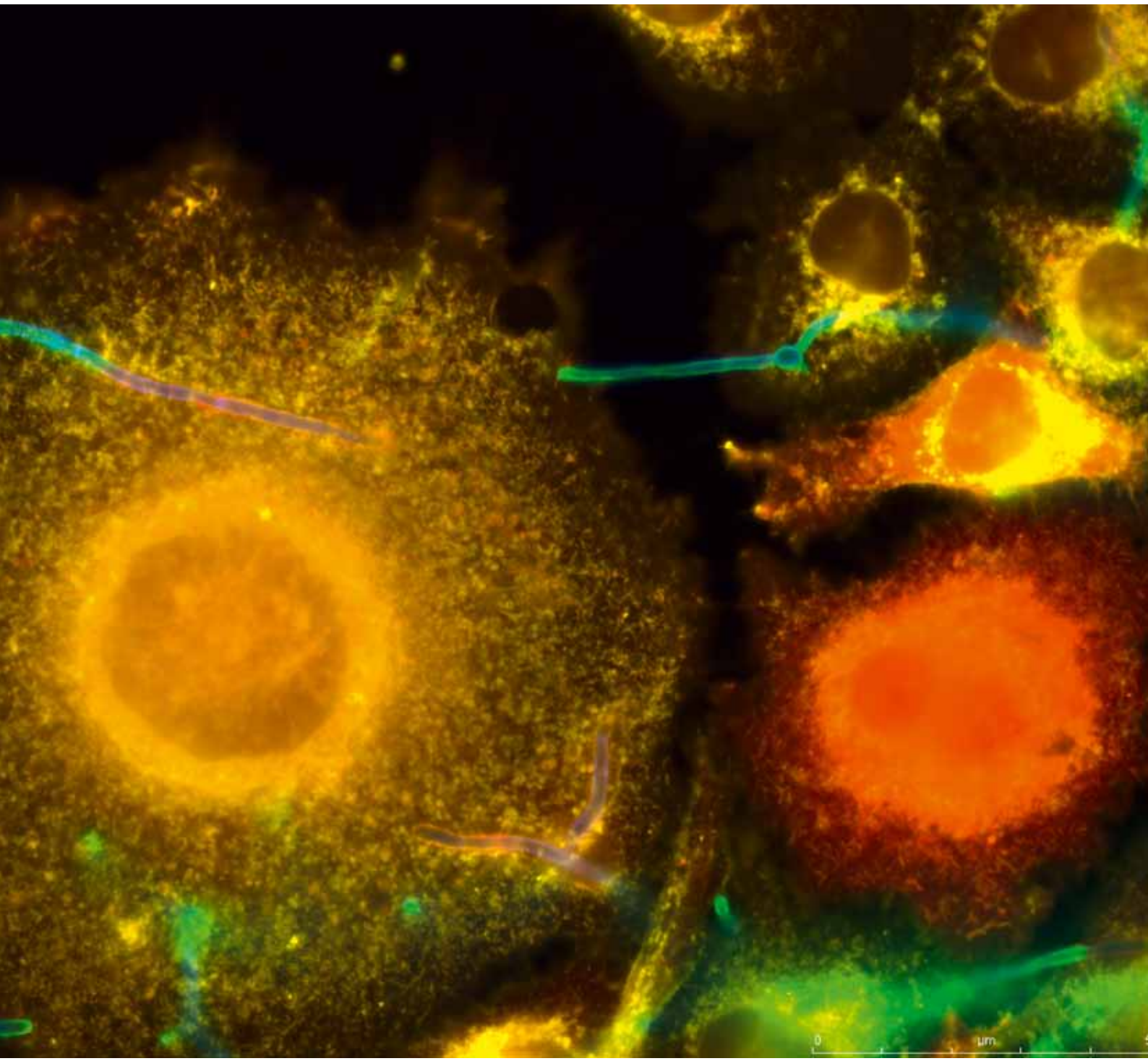
PhD student	Supervisor
Interactions of pathogenic microorganisms with hosts	
Hannes Eberhardt	Prof. Peter F. Zipfel
Tina Enghardt	PD Dr. Christine Skerka
Hoang Hoa Long	Prof. Ian T. Baldwin
Shanshan Luo	Prof. Peter F. Zipfel
François Mayer	Prof. Bernhard Hube
Shruthi Ramachandra	Prof. Bernhard Hube
Jennifer Sneed	Prof. Georg Pohnert
Krisztina Truta-Feles	Prof. Johannes Norgauer
Katrin Volling	Prof. Hans Peter Saluz
Arne Weinhold	Prof. Ian T. Baldwin
Hangxing Yu	Prof. Eberhard Straube

Institute	Project title
Leibniz Institute for Natural Product Research and Infection Biology – Hans Knöll Institute Department of Infection Biology	The role of CFHR proteins in human autoimmune diseases
Leibniz Institute for Natural Product Research and Infection Biology – Hans Knöll Institute Department of Infection Biology	Complement evasion of human pathogenic microorganisms
Max Planck Institute for chemical Ecology Department of Molecular Ecology	Characterizing and analyzing the ecological consequences of the plant-endophyte interactions in *Solanum nigrum* and *Nicotiana attenuata*
Leibniz Institute for Natural Product Research and Infection Biology – Hans Knöll Institute Department of Infection Biology	*Candida albicans*-host interaction, the many faces of *Candida* Pra1
Leibniz Institute for Natural Product Research and Infection Biology – Hans Knöll Institute Department of Microbial Pathogenicity Mechanisms	Identification and characterisation of infection-associated genes in *Candida albicans*
Leibniz Institute for Natural Product Research and Infection Biology – Hans Knöll Institute Department of Microbial Pathogenicity Mechanisms	Host pathogen interactions of human-pathogenic yeast
Friedrich Schiller University Jena Institute of Inorganic and Analytical Chemistry	Effects of the green alga *Dictyosphaeria ocellata* on its surrounding bacterial community
Jena University Hospital Clinic for Dermatology	Function of phosphatidylinositol-3-kinase-γamma and SH2-containing inositol-5-phosphatase-1 in innate immunity
Leibniz Institute for Natural Product Research and Infection Biology – Hans Knöll Institute Department of Cell and Molecular Biology	The human pathogenic fungus *Aspergillus fumigatus* inhibits apoptosis in alveolar macrophages
Max Planck Institute for chemical Ecology Department of Molecular Ecology	Microbial interactions relevant for the fitness of *Nicotiana attenuata* in the native environment
Jena University Hospital Institute for Medical Microbiology	Modulation of host death by *Chlamydia trachomatis* – the role of the *Chlamydia*-specific protease CPAF

Continuation →

PhD student	Supervisor
Networks, interactions and their analysis	
Daniela Albrecht	PD Dr. Reinhard Guthke
Fabian Horn	PD Dr. Reinhard Guthke
Christian Hummert	PD Dr. Reinhard Guthke
Radhika Jain	Prof. Axel A. Brakhage
Mohan Karthik Mohan	Prof. Maria Mittag
Sebastian Müller	PD Dr. Reinhard Guthke
Felicitas Schöbel	Dr. Matthias Brock
Stefanie Seitz	Prof. Maria Mittag
Andreas Thywissen	Prof. Axel A. Brakhage
Yong Qiang Wang	Prof. Günter Theißen

Institute	Project title
Leibniz Institute for Natural Product Research and Infection Biology – Hans Knöll Institute Research Group Systems Biology and Bioinformatics	Integration of transcriptome and proteome data from human-pathogenic fungi
Leibniz Institute for Natural Product Research and Infection Biology – Hans Knöll Institute Research Group Systems Biology and Bioinformatics	Prediction of gene regulatory networks Iinvolved in the differentiation, secondary metabolism and cross talk of *Aspergillus nidulans*
Leibniz Institute for Natural Product Research and Infection Biology – Hans Knöll Institute Research Group Systems Biology and Bioinformatics	Error Ccorrection for the integration of proteome and transcriptome data
Leibniz Institute for Natural Product Research and Infection Biology – Hans Knöll Institute Department of Molecular and Applied Microbiology	Map kinase MpkA regulated cell wall integrity signaling pathway in *Aspergillus fumigatus*
Friedrich Schiller University Jena Institute of General Botany and Plant Physiology	An insertional mutagenesis approach to understand temperature entrainment of the circadian clock in *Chlamydomonas reinhardtii*
Leibniz Institute for Natural Product Research and Infection Biology – Hans Knöll Institute Research Group Systems Biology and Bioinformatics	Integrated genome-wide data analysis by ensemble learning methods to understand infection processes
Leibniz Institute for Natural Product Research and Infection Biology – Hans Knöll Institute Junior Research Group Microbial Biochemistry and Physiology	Lysine biosynthesis in *Aspergillus fumigatus*: virulence studies and biochemical analysis
Friedrich Schiller University Jena Institute of General Botany and Plant Physiology	Functional characterization of molecular components of the circadian clock of the green alga *Chlamydomonas reinhardtii*
Leibniz Institute for Natural Product Research and Infection Biology – Hans Knöll Institute Department of Molecular and Applied Microbiology	Molecular mechanisms of the interaction between *Aspergillus fumigatus* and alveolar macrophages
Friedrich Schiller University Jena Department of Genetics	The evolutionary origin of floral quartets: clues from molecular interactions of orthologues of floral homeotic proteins from the gymnosperm *Gnetum gnemon*



Appendix

Appendix

Peer Reviewed Articles 2008
Originalarbeiten 2008

Department Biomolecular Chemistry

Christian B, Below A, Dressler N, **Scheibner O**, Luckas B, Gerdts G (2008) Are spirolides converted in biological systems? – A study. *Toxicon* 51, 934-940.

Fritzsche K, **Ishida K**, **Hertweck C** (2008) Orchestration of discoid polyketide cyclization in the resistomycin pathway. *J Am Chem Soc* 130, 8307-8316.

Gebhardt P, Crumbliss AL, Miller MJ, Moellmann U (2008) Synthesis and biological activity of saccharide based lipophilic siderophore mimetics as potential growth promoters for mycobacteria. *Biometals* 21, 41-51.

Han L, Huang X, Dahse HM, Moellmann U, **Grabley S**, Lin W, **Sattler I** (2008) New abietane diterpenoids from the mangrove *Avicennia marina*. *Planta Med* 74, 432-437.

Huang H, He J, Niu X, Menzel K-D, Dahse HM, **Grabley S**, Fiedler P, **Sattler I***, **Hertweck C*** (2008) Benzopyrenomycin, a cytotoxic bacterial polyketide metabolite with an unprecedented benzo[a]pyrene-type carbacyclic ring system. *Angew Chem Int Ed* 47, 3995-3998.
* equal contribution.

Jahanshahi M, **Partida-Martinez LP**, Hajizadeh S (2008) Preparation and evaluation of polymer-coated adsorbents for the expanded bed recovery of protein products from particulate feedstocks. *J Chromatogr A* 1203, 13-20.

Li L, **Sattler I**, Deng Z, Groth I, Walther G, Menzel K-D, Peschel G, **Grabley S**, Lin W (2008) A-seco-oleane-type triterpenes from *Phomopsis* sp. (strain HKI0458) isolated from the mangrove plant *Hibiscus tiliaceus*. *Phytochemistry* 69, 511-517.

Li LY, Ding Y, Groth I, Menzel K-D, Peschel G, Voigt K, Deng ZW, **Sattler I**, Lin WH (2008) Pyrrole and indole alkaloids from an endophytic *Fusarium incarnatum* (HKI00504) isolated from the mangrove plant *Aegiceras corniculatum*. *J Asian Nat Prod Res* 10, 765-770.

Li Y-Q, Huang XS, **Ishida K**, Maier A, Kelter G, Jiang Y, Peschel G, Menzel K-D, Li M-G, Wen M-L, Xu L-H, **Grabley S**, Fiebig H-H, Jiang C-L, **Hertweck C***, **Sattler I*** (2008) Plasticity in gilvocarcin-type C-glycoside pathways: discovery and antitumoral evaluation of polycarcin V from *Streptomyces polyformus*. *Org Biomol Chem* 6, 3601-3605.
* equal contribution.

Mies KA, **Gebhardt P**, Moellmann U, Crumbliss AL (2008) Synthesis, siderophore activity and iron(III) chelation chemistry of a novel mono-hydroxamate, bis-catecholate siderophore mimic. *J Inorg Biochem* 102, 850-861.

Muggia L, **Schmitt I**, Grube M (2008) Purifying selection is a prevailing motif in the evolution of ketoacyl synthase domains of polyketide synthases from lichenized fungi. *Mycol Res* 112, 277-288.

Nguyen T*, **Ishida K***, Jenke-Kodama H, Dittmann E, Gurgui C, Hochmuth T, Taudien S, Platzer M, **Hertweck C**, Piel J (2008) Exploiting the mosaic structure of trans-acyl transferase polyketide synthases for natural product discovery and pathway dissection. *Nature Biotechnol* 26, 225-233.
* equal contribution.

Niu X, Dahse HM, Menzel K-D, Lozach O, Walther G, Meijer L, **Grabley S**, **Sattler I** (2008) Butyrolactone I derivatives from *Aspergillus terreus* carrying an unusual sulfate moiety. *J Nat Prod* 71, 689-692.

Partida-Martinez LP, Ruechel R, Dannoui E, **Hertweck C** (2008) Lack of evidence for endofungal bacteria in the development of rhizopus-borne zygomycoses in humans. *Mycoses* 51, 266-269.

Richter MEA, **Traitcheva N**, Knuepfer U, **Hertweck C** (2008) Sequential asymmetric polyketide heterocyclization catalyzed by a single cytochrome P450 monooxygenase (AurH). *Angew Chem Int Ed* 47, 8872-8875.

Schmitt I, Kautz S Lumbsch HT (2008) 6-MSAS-like polyketide synthase genes occur in lichenized ascomycetes. *Mycol Res* 112, 289-296.

Schmitt I, **Partida-Martinez LP**, **Winkler R**, Voigt K, Einax A, Woestemeyer J, **Hertweck C** (2008) Evolution of host-resistance in a toxin-producing fungal-bacterial mutualism. *ISME J* 2, 632-641.

Wangun HVK, **Ishida K**, **Hertweck C** (2008) Epicoccalone, a coumarin-type chymotrypsin inhibitor, and isobenzofuran congeners from an *Epicoccum* sp. associated with a tree fungus. *Eur J Org Chem* 22, 3781-3784.

Werneburg M, **Hertweck C** (2008) Chemoenzymatic total synthesis of the antiproliferative polyketide (+)-R-aureothin. *ChemBioChem* 9, 2064-2066.

Ziemert N*, **Ishida K***, Liaimer A, **Hertweck C***, Dittmann E* (2008) Ribosomal synthesis of tricyclic depsipeptides in bloom-forming cyanobacteria. *Angew Chem Int Ed* 47, 7756-7759.
* equal contribution.

Ziemert N*, **Ishida K***, Quillardet P, Bouchier C, **Hertweck C**, Tandeau de Marsac N, Dittmann E (2008) Microcyclamide biosynthesis in two strains of *Microcystis aeruginosa*: from structure to genes and vice versa. *Appl Environ Microbiol* 74, 1791-1797.
* equal contribution.

Department Cell and Molecular Biology

Carlsohn M, Groth I, **Saluz HP**, Stackebrandt E (2008) *Fodinicola feengrottensis* gen. nov. sp. nov., a novel actinomycete isolated from a medieval mine. *IJSEM* 58, 1529-1536.

Gebhardt P, Crumbliss AL, Miller MJ, Moellmann U (2008) Synthesis and biological activity of saccharide based lipophilic siderophore mimetics as potential growth promoters for mycobacteria. *Biometals* 21, 41-51.

Grote D, **Haenel F**, Dahse HM, Seifert K (2008) Capnellenes from the soft coral *Dendronephthya rubeola*. *Chemistry & Biodiversity* 5, 1683-1693.

Ibanez A, Schueler T, Moeller R, Fritzsche W, **Saluz HP,** Svatos A (2008) DNA detection using a novel triple read-out optical/AFM/MALDI planar microwell plastic chip. *Analytical Chemistry* 80, 5892-5898.

Iqbal J, **Haenel F**, Ruryk A, **Limmon G**, **Tretiakov A**, Duerst M, **Saluz HP** (2008) Fabrication and evaluation of a sequence specific oligonucleotide micro-chip for molecular genotyping. *IJMM* 26, 13-20.

Mies KA, **Gebhardt P**, Moellmann U, Crumbliss AL (2008) Synthesis, siderophore activity and iron(III) chelation chemistry of a novel mono-hydroxamate, bis-catecholate siderophore mimic: N(alpha),-N(epsilon)-Bis[2,3-dihydroxybenzoyl]-l-lysyl-(gamma-N-methyl-N-hydroxyamido)-L-glutamic acid. *J Inorg Biochem* 102, 850-861.

Moeller R, Schueler T, **Guenther S**, **Carlsohn M**, **Munder T**, Fritzsche W (2008) Electrical DNA-chip-based identification of different species of the genus *Kitasatospora*. *Appl Microbiol Biotechnol* 77, 1181-1188.

Schmidt U, **Wollmann Y**, **Franke C**, Grosse F, **Saluz HP, Haenel F** (2008) Characterization of the interaction between the human topoisomerase IIb binding protein 1 (TopBP1) and the cell division cycle protein 45 (Cdc45). *Biochem J* 409, 169-177.

Tretiakov A, Erfurth F, Nyuyki B, **Mrotzek G**, Schmidt WD, Fassler D, **Saluz HP** (2008) Two-laser, large field hyperspectral microarray scanner for the analysis of multicolour microarrays. *Analytical Chemistry* 80, 7706-7713.

Department Infection Biology

Barthel D, Schlitzer M, Pradel G (2008) Telithromycin and quinupristin-dalfopristin induce delayed death in *Plasmodium falciparum*. *Antimicrob Agents Chemother* 52, 774-777.

Behnsen J, **Hartmann A**, Schmaler J, Gehrke A, Brakhage AA, **Zipfel PF** (2008) The opportunistic human pathogenic fungus *Aspergillus fumigatus* evades the host complement system. *Inf Immun* 76, 820-827.

Castelletti F, Donadelli R, Banterla F, Hildebrandt F, **Zipfel PF**, Bresin E, Otto E, **Skerka C**, Renieri A,

Todeschini M, Caprioli J, Caruso MR, Artuso R, Remuzzi G, Noris M (2008) Mutations in FN1 cause glomerulopathy with fibronectin deposits. *Proc Natl Acad Sci USA* 105, 2538-2543.

Grote D, Haenel F, **Dahse HM**, Seifert K (2008) Capnellenes from the soft coral *Dendronephthya rubeola*. *Chemistry & Biodiversity* 5, 1683-1693.

Habbig S, **Mihatsch MJ**, **Heinen S**, Beck B, Emmel M, **Skerka C**, Kirschfink M, Hoppe B, **Zipfel PF**, Licht C (2008) C3 deposition glomerulopathy due to a funtional factor H defect. *Kidney Int* 75, 1230-1234.

Hallstroem T, **Zipfel PF**, Blom AM, **Lauer N**, Forsfren A, Riesbeck K (2008) *Haemophilus influenzae* interacts with the human complement inhibitor factor H. *J Immunol* 181, 537-545.

Han L, Huang X, **Dahse HM**, Moellmann U, Grabley S, Lin W, Sattler I (2008) New abietane diterpenoids from the mangrove *Avicennia marina*. *Planta Medica* 74, 432-437.

Haupt K, Kraiczy K, Wallich R, Brade V, **Skerka C**, **Zipfel PF** (2008) FHR-1, an additional human plasma protein, binds to complement regulator-acquiring surface proteins of *Borrelia burgdorferi*. *Int J Med Microbiol* 298, 287-291.

Haupt K, **Reuter M**, van den Elsen J, Burman J, **Haelbich S**, **Richter H**, **Skerka C**, **Zipfel PF** (2008) The *Staphylococcus aureus* protein Sbi acts as a complement inhibitor and forms a tripartite complex with host complement factor H and C3b. *PloS Pathog* 4, e1000250.

Huang X, He J, Niu X, Menzel K-D, **Dahse HM**, Grabley S, Fiedler H-P, Sattler I, Hertweck C (2008) Benzopyrenomycin, a cytotoxic bacterial polyketide metabolite with an benzo[a]pyrene-type carbocyclic ring system. *Angew Chem Int Ed* 47, 3995-3998.

Józsi M, Licht C, Strobel S, **Zipfel SLH**, **Richter H**, **Heinen S**, **Zipfel PF**, **Skerka C** (2008) Factor H autoantibodies in atypical Hemolytic Uremic Syndrome correlate with CFHR1/CFHR3 deficiency. *Blood* 111, 1512-1514.

Kohring K, Wiesner J, Altenkaemper M, Sakowski J, Silber K, Hillebrecht A, Haebel P, **Dahse HM**, Ortmann R, Jomaa H, Klebe G, Schlitzer M (2008) Development of benzophenone-based farnesyltransferase inhibitors as novel anti-malarials. *Chem Med Chem* 3,1217-1231.

Koziolek MJ*, **Zipfel PF***, **Skerka C**, Vasko R, Groene EF, Mueller GA, Strutz F (2008) Chronic course of a haemolytic uraemic syndrome caused by a deficiency of factor H related proteins (CFHR1 and CFHR3). *Kidney International* 74, 383-388.
* equal contribution.

Kraiczy P, Schreiber J, **Skerka C**, **Haupt K**, Brade V, Wallich R, **Zipfel PF** (2008) Assessment of the regions within complement regulator-acquiring surface protein (CRASP)-2 of *Borrelia burgdorferi* required for interaction with host immune regulators FHL-1 and factor H. *Int J Med Microbiol* 298, 268-271.

Krchnák V, Moellmann U, **Dahse HM**, Miller MJ (2008) Solid-supported nitroso hetero Diels-Alder reactions. 1. Acylnitroso dienophiles: Scope and limitations. *J Comb Chem* 10, 94-103.

Krchnák V, Moellmann U, **Dahse HM**, Miller MJ (2008) Solid-supported nitroso hetero Diels-Alder reactions. 2. Arylnitroso dienophiles: Scope and limitations. *J Comb Chem* 10, 104-111.

Krchnák V, Moellmann U, **Dahse HM**, Miller MJ (2008) Solid-supported nitroso hetero Diels-Alder reactions. 3. Acid-mediated transformation of cycloadducts by scission of the oxazine C-O bonds. *J Comb Chem* 10, 112-117.

Krchnák V, Waring KR, Noll BC, Moellmann U, **Dahse HM**, Miller MJ (2008) Evolution of natural product scaffolds by acyl- and arylnitroso hetero Diels-Alder reactions: New chemistry on piperine. *J Org Chem* 73, 4559-4567.

Niu X, **Dahse HM**, Menzel K-D, Lozach O, Walther G, Meijer L, Grabley S, Sattler I (2008) Butyrolactone I derivatives from *Aspergillus terreus* carrying an unusual sulfate moiety. *J Nat Prod* 71, 689-692.

Rossmann E, Kraiczy P, Herzberger P, **Skerka C**, Kirschfink M, Simon MM, **Zipfel PF**, Wallich R (2008) BhCRASP-1 of the relapsing fever spirochete *Borrelia hermsii* is a factor H- and plasminogen-binding protein. *Int J Med Microbiol* 298, 272-283.

Schroeder H, Skelly P, **Zipfel PF**, Losson B, Vanderplasschen A (2008) Subversion of complement by hematophagous parasites. *Dev Comp Immunol* 33, 5-13.

Siegel C, Herzberger P, **Skerka C**, Brade V, Fingerle V, Schulte-Spechtel U, Wilske B, **Zipfel PF**, Wallich R, Kraiczy P (2008) Binding of complement regulatory protein factor H enhances serum resistance of *Borrelia spielmanii* sp. nov. *Int J Med Microbiol* 298, 292-294.

Siegel C, Schreiber J, **Haupt K**, **Skerka C**, Brade V, Simon MM, Stevenson B, Wallich R, **Zipfel PF**, Kraiczy P (2008) Definition of ligand-binding sites in the *Borrelia burgdorferi* virulence-associated BbCRASP-2 (CspZ) protein required for interactions with the human immune regulators factor H and FHL-1. *J Biol Chem* 283, 34855-34863.

Stahl AI, Vaziri-Sani F, **Heinen S**, Kristoffersson AC, Gydell KH, Raafat R, Gutierrez A, Beringer O, **Zipfel PF**, Karpman D (2008) Factor H dysfunction in patients with atypical Hemolytic Uremic Syndrome contributes to complement deposition on platelets and their activation. *Blood* 111, 5307-5315.

Zipfel PF, **Hallstroem T**, Hammerschmidt S, **Skerka C** (2008) The complement fitness factor H: role in human diseases and for immune escape of pathogens, like pneumococci. *Vaccine* 26, 67-74.

Department Microbial Pathogenicity Mechanisms

Almeida RS, **Brunke S**, **Albrecht A**, Thewes S, Laue M, Edwards JE, Filler SG Jr, **Hube B** (2008) The hyphal-associated adhesin and invasin Als3 of *Candida albicans* mediates iron acquisition from host ferritin. *PLoS Pathogens* 4, e1000217.

Bader O, Krauke Y, **Hube B** (2008) Processing of predicted substrates of fungal Kex2 proteinases from *Candida albicans*, *C. glabrata*, *Saccharomyces cerevisiae* and *Pichia pastoris*. *BMC Microbiol* 8, 116.

Hennig-Pauka I, Baltes N, **Jacobsen I**, Stratmann-Selke J, Gerlach GF, Selbitz HJ, Waldmann KH (2008) Study of the virulence of *Actinobacillus pleuropneumoniae* in finishing pigs as a basis for vaccination development. *Berl Munch Tierarztl Wochenschr* 121, 189-197.

Mora-Montes HM, Bader O, López-Romero E, Zinker S, Ponce-Noyola P, **Hube B**, Gow NAR, Flores-Carreón A (2008) Kex2 protease converts the endoplasmic reticulum a1,2-mannosidase of *Candida albicans* into a soluble cytosolic form. *Microbiology* 154, 3782-3794.

Mota Júnior AO, Malavazi I, Soriani FM, Heinekamp T, **Jacobsen I**, Brakhage AA, Savoldi M, Goldman MH, da Silva Ferreira ME, Goldman GH (2008) Molecular characterization of the *Aspergillus fumigatus* NCS-1 homologue, NcsA. *Mol Genet Genomics* 280, 483-495.

Naglik JR, Moyes D, Makwana J, Kanzaria P, Tsichlaki E, Weindl G, Tappuni AR, Rodgers CA, Woodman AJ, Challacombe SJ, Schaller M, **Hube B** (2008) Quantitative expression of the *Candida albicans* secreted aspartyl proteinase gene family in human oral and vaginal candidiasis. *Microbiology* 154, 3266-3280.

Ozegowski JH, Presselt N, **Haertl A**, Bocker T, Saenger J, Schmidt A, Willing K, Mueller PJ (2008) Anti-atherosclerotic effect of microbial hyaluronate lyase from group B streptococci. *Pharmazie* 63, 601-605.

Schrettl M, Kim HS, Eisendle M, Kragl C, Nierman WC, Heinekamp T, Werner ER, **Jacobsen I**, Illmer P, Yi H, Brakhage AA, Haas H (2008) SreA-mediated iron regulation in *Aspergillus fumigatus*. *Mol Microbiol* 70, 27-43.

Thewes S, Moran GP, Magee BB, Schaller M, Sullivan DJ, **Hube B** (2008) Phenotypic screening, transcriptional profiling, and comparative genomic analysis of an invasive and non-invasive strain of *Candida albicans*. *BMC Microbiology* 8, 187.

Valiante V, Heinekamp T, Jain R, **Haertl A**, Brakhage AA (2008) The mitogen activated proteine kinase MpkA of *Aspergillus fumigatus* regulates cell wall signaling and oxidative stress response. *Fungal Genet Biol* 45, 618-627.

Wozniok I, Hornbach A, Schmitt C, Frosch M, Einsele H, **Hube B**, Loeffler J, Kurzai O (2008) Induction of ERK kinase signalling triggers morphotype specific killing of *Candida albicans* filaments by human neutrophils. *Cellular Microbiology* 10, 807-820.

Department Molecular and Applied Microbiology

Behnsen J, Hartmann A, **Schmaler J**, **Gehrke A**, **Brakhage AA**, Zipfel PF (2008) The opportunistic human pathogenic fungus *Aspergillus fumigatus* evades the host complement

system. *Infection and Immunity* 76, 820-827.

Duerrschmid K, Heischer H, **Schmidt-Heck W**, Hrebicek T, **Guthke R**, Rizzi A, Bayer K (2008) Monitoring of transcriptome and proteome profiles to investigate the cellular response of *E. coli* towards recombinant gene expression under defined chemostat conditions. *J Biotechnol* 135, 34-44.

Erhard M, Hipler UC, **Burmester A**, **Brakhage AA**, Woestemeyer J (2008) Identification of clinical dermatophyte isolates by MALDI-TOF mass spectrometry. *European Journal of Dermatology* 17, 356-361.

Fennell KA, **Moellmann U**, Miller MJ (2008) Syntheses and biological activity of amamistatin B and analogs. *J Org Chem* 73, 1018-1024.

Freilich M, Patel CM, Wei M, Shafer D, Schleier P, **Hortschansky P**, Kompali R, Kuhn L (2008) Growth of new bone guided by implants in a murine calvarial model. *Bone* 43, 781-788.

Friedel M, **Nikolajewa S**, Suehnel S, Wilhelm T (2008) DiProDB: a database for dinucleotide properties. *Nucleic Acids Research*, 1-4.

Funke M, Buechler R, Mahobia V, Schneeberg A, **Ramm M**, Boland W (2008) Rapid hydrolysis of quorum-sensing molecules in the gut of *Lepidopteran larvae. Chembiochem* 9, 1953-1959.

Gebhardt P, Crumbliss AL, Miller MJ, **Moellmann U** (2008) Synthesis and biological activity of saccharide based lipophilic siderophore mimetics as potential growth promoters for mycobacteria. *Biometals* 21, 41-51.

Glahn F, **Schmidt-Heck W**, Zellmer S, **Guthke R**, Wiese J, Golka K, Hergenroeder R, Degen GH, Lehmann T, Hermes M, Schormann W, Brulport M, Bauer A, Bedawy E, Gebhardt R, Hengstler JG, Foth H (2008) Cadmium, cobalt and lead cause stress response, cell cycle deregulation and increased steroid as well as xenobiotic metabolism in primary normal human bronchial epithelial cells which is coordinated by at least nine transcription factors. *Arch Toxicol* 82, 513-524.

Grosse C, **Heinekamp T**, **Kniemeyer O**, **Gehrke A**, **Brakhage AA** (2008) Protein kinase A regulates growth, development and pigmentation in *Aspergillus fumigatus. Applied and Environmental Microbiology* 74, 4923-4933.

Han L, Huang X, Dahse HM, **Moellmann U**, Grabley S, Lin W, Sattler I (2008) New abietane diterpenoids from the mangrove *Avicennia marina. Planta Medica* 74, 432-437.

Huber R, Hummert C, Gausmann U, Koczan D, **Guthke R**, Kinne RW (2008) Identification of intra-group, inter-individual, and gene-specific variances in mRNA expression profiles in the rheumatoid arthritis synovial membrane. *Arthritis Res Ther* 10, R98.

Ibrahim-Granet O, Dubourdeau M, Latge JP, Ave P, Huerre M, **Brakhage AA**, Brock M (2008) Methylcitrate synthase from *Aspergillus fumigatus* is essential for the manifestation of invasive aspergillosis. *Cell Microbiol* 10, 134-148.

Janus D, **Hortschansky P**, Kueck U (2008) Identification of a minimal cre1 promoter sequence promoting glucose-dependent gene expression in the beta-lactam producer *Acremonium chrysogenum. Curr Genet* 53, 35-48.

Klimešová V, Kočí J, Waisser K, Kaustová J, **Moellmann U** (2008) Preparation and *in vitro* evaluation of benzylsulfanyl benzoxazole derivatives as potential antituberculosis agents. *Eur J Med Chem* 44, 2286-2293.

Koczan D, Drynda S, Hecker M, Drynda A, **Guthke R**, Kekow J, Thiesen HJ (2008) Molecular discrimination of responders and non-responders to anti-TNF-alpha therapy in rheumatoid arthritis by etanercept. *Arthritis Res Ther* 10, R50.

Konter J, **Moellmann U**, Lehmann J (2008) NO donors, part 17: synthesis and antimicrobial activity of novel ketoconazole-NO-donor hybrid compounds. *Bioorg Med Chem* 16, 8294-8300.

Krchnák V, **Moellmann U**, Dahse HM, Miller MJ (2008) Solid-supported nitroso hetero Diels-Alder reactions. 1. Acylnitroso dienophiles: scope and limitations, *J Comb Chem* 10, 94-103.

Krchnák V, **Moellmann U**, Dahse HM, Miller MJ, (2008) Solid-supported nitroso hetero Diels-Alder reactions. 3. Acid-mediated transformation of cycloadducts by scission of the oxazine C-O bonds. *J Comb Chem* 10, 112-117.

Krchnák V, **Moellmann U**, Dahse HM, Miller, MJ (2008) Solid-supported nitroso hetero Diels-Alder reactions. 2. Arylnitroso dienophiles: scope and limitations. *J Comb Chem* 10, 104-111.

Krchnák V, Waring KR, Noll BC, **Moellmann U**, Dahse HM, Miller MJ (2008) Evolution of natural product scaffolds by acyl- and arylnitroso hetero Diels-Alder reactions: new chemistry on piperine. *J Org Chem* 73, 4559-4567.

Mahboobi S, Eichhorn E, Winkler M, **Moellmann U** (2008) Antibacterial activity of a novel series of 3-bromo-4-(1H-3-indolyl)-2,5-dihydro-1H-2,5-pyrroledione derivatives - an extended structure activity relationship study. *J Med Chem* 43, 633-656.

Marchand C, Beutler JA, Wamiru A, Budihas S, **Moellmann U**, Heinisch L, Mellors JW, Legrice S, Pommier Y, (2008) Madurahydroxylactone derivatives as dual inhibitors of HIV-1 integrase and RNAse. *Antimicrob Agents Chemother* 52, 361-364.

Mies KA, Gebhardt P, **Moellmann U**, Crumbliss AL (2008) Synthesis, siderophore activity and iron(III) chelation chemistry of a novel mono-hydroxamate, bis-catecholate siderophore mimic: N(alpha),-N(epsilon)-Bis[2,3-dihydroxybenzoyl]-l-lysyl-(gamma-N-methyl-N-hydroxyamido)-L-glutamic acid. *J Inorg Biochem* 102, 850-861.

Mota AO Jr, Malavazi I, Soriani FM, **Heinekamp T**, Jacobsen I, **Brakhage AA**, Savoldi M, de Souza Goldman MH, da Silva Ferreira ME, Goldman GH (2008) Molecular characterization of the *Aspergillus fumigatus* NCS-1 homologue, NcsA. *Molecular Genetics and Genomics* 280, 483-495.

Schrettl M, Kim HS, Eisendle M, Kragl C, Niermann WC, **Heinekamp T**, Werner ER, Jacobsen I, Ilmer P, Yi H, **Brakhage AA**, Haas H (2008) SreA-mediated iron regulation in *Aspergillus fumigatus. Molecular Microbiology* 70, 27-43.

Shakèd M, Weissmueller K, Svoboda H, **Hortschansky P**, Nishino N, Woelfl S, Tucker KL (2008) Histone deacetylases control neurogenesis in embryonic brain by inhibition of BMP2/4 signaling. *PLoS ONE* 16, e2668.

Sproete P, Hynes MJ, **Hortschansky P**, **Scharf D**, **Wolke S**, **Brakhage AA** (2008) Evolution of fungal secondary metabolism genes: characterisation of the novel penicillin biosynthesis gene aatB of *Aspergillus nidulans*, the putative ancestor of aatA. *Molecular Microbiology* 70, 445-461.

Steinert S, Kroll TC, Taubert I, Pusch L, **Hortschansky P**, Hoeffken K, Woelfl S, Clement JH (2008) Differential expression of cancer-related genes by single and permanent exposure to bone morphogenetic protein 2. *J Cancer Res Clin Oncol* 134, 1237-1245.

Valiante V, **Heinekamp T**, **Jain R**, **Haertl A**, **Brakhage AA** (2008) The mitogen activated proteine kinase MpkA of *Aspergillus fumigatus* regulates cell wall signaling and oxidative stress response. *Fungal Genetics and Biology* 45, 618-627.

Wollbold J, **Guthke R**, Ganter B (2008) Constructing a knowledge base for gene regulatory dynamics by formal concept analysis methods. *Lecture Notes in Computer Sciences* 5147, 230-244.

Wortman JR, Gilsenan JM, Joardar V, Deegan J, Clutterbuck J, Andersen MR, Archer D, Bencina M, Braus G, Coutinho P, Doehren HV, Doonan J, Driessen AJ, Durek P, Espeso E, Fekete E, Flipphi M, Estrada CG, Geysens S, Goldman G, de Groot PW, Hansen K, Harris SD, **Heinekamp T**, Helmstaedt K, Henrissat B, Hofmann G, Homan T, Horio T, Horiuchi H, James S, Jones M, Karaffa L, Karányi Z, Kato M, Keller N, Kelly DE, Kiel JA, Kim JM, van der Klei IJ, Klis FM, Kovalchuk A, Kraševec N, Kubicek CP, Liu B, Maccabe A, Meyer V, Mirabito P, Miskei M, Mos M, Mullins J, Nelson DR, Nielsen J, Oakley BR, Osmani SA, Pakula T, Paszewski A, Paulsen I, Pilsyk S, Pócsi I, Punt PJ, Ram AF, Ren Q, Robellet X, Robson G, Seiboth B, van Solingen P, Specht T, Sun J, Taheri-Talesh N, Takeshita N, Ussery D, Vankuyk PA, Visser H, van de Vondervoort PJ, de Vries RP, Walton J, Xiang X, Xiong Y, Zeng AP, Brandt BW, Cornell MJ, van den Hondel CA, Visser J, Oliver SG, Turner G (2008) The 2008 update of the *Aspergillus nidulans* genome annotation: a community effort. *Fungal Genet Biol* 46, 2-13.

Junior Research Group Cellular Immunobiology

Józsi M, Licht C, **Strobel S**, Zipfel SHL, Richter H, Heinen S, Zipfel PF, Skerka C (2008) Factor H autoantibodies in atypical Hemolytic Uremic Syndrome correlate with CFHR1/CFHR3 deficiency. *Blood* 111, 1512-1514.

Junior Research Group Microbial Biochemistry and Physiology

Brock M, Jouvion G, Droin-Bergère S, Dussurget O, Nicola MA, Ibrahim-Granet O (2008) Bioluminescent *Aspergillus fumigatus*: a new tool for drug efficiency testing and *in vivo* monitoring of invasive aspergillosis. *Appl Environ Microbiol* 74, 7023-7035.

Fleck CB, **Brock M** (2008) Characterization of an acyl-CoA:carboxylate CoA-transferase from *Aspergillus nidulans* involved in propionyl-CoA detoxification. *Mol Microbiol* 68, 642-656.

Ibrahim-Granet O, Dubourdeau M, Latgé J-P, Ave P, Huerre M, Brakhage AA, **Brock M** (2008) Methylcitrate synthase from *Aspergillus fumigatus* is essential for manifestation of invasive aspergillosis. *Cell Microbiol* 10, 34-48.

Junior Research Group Fundamental Molecular Biology of Pathogenic Fungi

Degel B, **Staib P**, Rohrer S, Scheiber J, Martina E, Buechold C, Baumann K, Morschhaeuser J, Schirmeister T (2008) Cis-configured aziridines are new pseudo-irreversible dual-mode inhibitors of *Candida albicans* secreted aspartic protease 2. *ChemMedChem* 3, 302-315.

Staib P, **Lermann U**, Blass-Warmuth J, Degel B, Wuerzner R, Monod M, Schirmeister T, Morschhaeuser J (2008) Tetracycline-inducible expression of individual secreted aspartic proteases in *Candida albicans* allows isoenzyme-specific inhibitor screening. *Antimicrob Agents Chemother* 52, 146-156.

Zaugg C, Jousson O, Léchenne B, **Staib P,** Monod M (2008) *Trichophyton rubrum* secreted and membrane-associated carboxypeptidases. *Int J Med Microbiol* 298, 669-682.

Junior Research Group Secondary Metabolism of Predatory Bacteria

McGlinchey RP, **Nett M**, Eustaquio AS, Asolkar RN, Fenical W, Moore BS (2008) Engineered biosynthesis of antiprotealide and other unnatural salinosporamide proteasome inhibitors. *J Am Chem Soc* 130, 7822-7823.

McGlinchey RP, **Nett M**, Moore BS (2008) Unraveling the biosynthesis of the sporolide cyclohexenone building block. *J Am Chem Soc* 130, 2406-2407.

Research Group Fungal Septomics

Haupt K, Kraiczy P, Wallich R, Brade V, Skerka C, Zipfel PF (2008) FHR-1, an additional human plasma protein, binds to complement regulator-acquiring surface proteins of *Borrelia burgdorferi. Int J Med Microbiol* 298, 287-291.

Haupt K, Reuter M, van den Elsen J, Burman J, Haelbich S, Richter J, Skerka C, Zipfel PF (2008) The *Staphylococcus aureus* protein Sbi acts as a complement inhibitor and forms a tripartite complex with host complement factor H and C3b. *PLoS Pathog* 4, e1000250.

Kraiczy P, Schreiber J, Skerka C, **Haupt K**, Brade V, Wallich R, Zipfel PF (2008) Assessment of the regions within complement regulator-acquiring surface protein (CRASP)-2 of *Borrelia burgdorferi* required for interaction with host immune regulators FHL-1 and factor H. *Int J Med Microbiol* 298, 268-271.

Mezger M, Kneitz S, Wozniok I, **Kurzai O**, Einsele H, Loeffler J (2008) Proinflammatory response of immature human dendritic cells is mediated by dectin-1 after exposure to *Aspergillus fumigatus* germ tubes. *J Infect Dis* 197, 924-931.

Mezger M, Wozniok I, Blockhaus C, **Kurzai O**, Hebart H, Einsele H, Loeffler J (2008) Impact of mycophenolic acid on the functionality of human polymorphonuclear neutrophils and dendritic cells during interaction with *Aspergillus fumigatus. Antimicrob Agents Chemother* 52, 2644-2646.

Restle A, Faerber M, Baumann C, **Boehringer M**, Scheidtmann KH, Mueller-Tidow C, Wiesmueller L (2008) Dissecting the role of p53 phosphorylation in homologous recombination provides new clues for gain-of-function mutants. *Nucleic Acids Res* 36, 5362-5375.

Schoen C, Blom J, Claus H, Schramm-Glueck A, Brandt P, Mueller T, Goesmann A, Joseph B, Konietzny S, **Kurzai O**, Schmitt C, Friedrich T, Linke B, Vogel U, Frosch M (2008) Whole-genome comparison of disease and carriage strains provides insights into virulence evolution in *Neisseria meningitidis. Proc Natl Acad Sci* USA 105, 3473-3478.

Siegel C, Schreiber J, **Haupt K**, Skerka C, Brade V, Simon MM, Stevenson B, Wallich R, Zipfel PF, Kraiczy P (2008) Deciphering the ligand-binding sites in the *Borrelia burgdorferi* complement regulator-acquiring surface protein 2 required for interactions with the human immune regulators factor H and factor H-like protein 1. *J Biol Chem* 283, 34855-34863.

Valenza G, Strasen J, Schaefer F, Frosch M, **Kurzai O**, Abele-Horn M (2008) Evaluation of new colorimetric vitek 2 yeast identification card by use of different source media. *J Clin Microbiol* 46, 3784-3787.

Villwock A, Schmitt C, Frosch M, **Kurzai O** (2008) Capsule acetylation does not impair recognition of serogroup C, W-135 and Y meningococci by human dendritic cells. *Int J Med Microbiol* 298, 591-597.

Villwock A, Schmitt C, Schielke S, Frosch M, **Kurzai O** (2008) Recognition via the class A scavenger receptor modulates cytokine secretion by human dendritic cells after contact with *Neisseria meningitidis. Microbes Infect* 10, 1158-1165.

Wozniok I, Hornbach A, Schmitt C, Frosch M, Einsele H, Hube B, Loeffler J, **Kurzai O** (2008) Induction of ERK-kinase signalling triggers morphotype-specific killing of *Candida albicans* filaments by human neutrophils. *Cell Microbiol* 10, 807-820.

Research Group Pharmaceutical Biology

Misiek M, **Hoffmeister D** (2008) mRNA processing sites involved in intron splicing of *Armillaria* natural product genes. *Mycol Res* 112, 216-224.

Schneider P, Bouhired S, **Hoffmeister D** (2008) Characterization of the atromentin biosynthesis genes and enzymes in the homobasidiomycete *Tapinella panuoides. Fungal Genet Biol* 45, 1487-1496.

Schneider P, Weber M, **Hoffmeister D** (2008) The *Aspergillus nidulans* enzyme TdiB catalyzes prenyltransfer to the precursor of bioactive asterriquinones. *Fungal Genet Biol* 45, 302-309.

Research Group Systems Biology / Bioinformatics

Duerrschmid K, Heischer H, **Schmidt-Heck W**, Hrebicek T, **Guthke R**, Rizzi A, Bayer K (2008) Monitoring of transcriptome and proteome profiles to investigate the cellular response of *E. coli* towards recombinant gene expression under defined chemostat conditions. *J Biotechnol* 135, 34-44.

Glahn F, **Schmidt-Heck W**, Zellmer S, **Guthke R**, Wiese J, Golka K, Hergenroeder R, Degen GH, Lehmann T, Hermes M, Schormann W, Brulport M, Bauer A, Bedawy E, Gebhardt R, Hengstler JG, Foth H (2008) Cadmium, cobalt and lead cause stress response, cell cycle deregulation and increased steroid as well as xenobiotic metabolism in primary normal human bronchial epithelial cells which is coordinated by at least nine transcription factors. *Arch Toxicol* 82, 513-524.

Huber R, Hummert C, Gausmann U, Koczan D, **Guthke R**, Kinne RW (2008) Identification of intra-group, inter-individual, and gene-specific variances in mRNA expression profiles in the rheumatoid arthritis synovial membrane. *Arthritis Res Ther* 10, R98.

Koczan D, Drynda S, Hecker M, Drynda A, **Guthke R**, Kekow J, Thiesen HJ (2008) Molecular discrimination of responders and non-responders to anti-TNF-alpha therapy in rheumatoid arthritis by etanercept. *Arthritis Res Ther* 10, R50.

Lovas A, **Radke D**, **Albrecht D**, Yilmaz ZB, **Moeller U**, Habenicht AJR, Weih F (2008) Differential RelA- and RelB-dependent gene transcription in LTβR-stimulated mouse embryonic fibroblasts. *BMC Genomics* 9, 606.

Moeller U (2008) Improved automatic control of fuzzy C-means clustering of high-dimensional data based on new methods for optimising the fuzziness parameter. *BIRD08 Schriftenreihe Informatik* 26, 85-94.

Shelest E (2008) Transcription factors in fungi - minireview. *FEMS Microbiology Letters* 286, 145-151.

Sproete P, Hynes MJ, Hortschansky P, **Shelest E**, Scharf DH, Wolke SM, Brakhage AA (2008) Identification of the novel penicillin biosynthesis gene aatB of *Aspergillus nidulans* and its putative evolutionary relationship to this fungal secondary metabolism gene cluster. *Mol Microbiol* 70, 445-461.

Wollbold J, **Guthke R**, Ganter B (2008) Constructing a knowledge base for gene regulatory dynamics by formal concept analysis methods. *Lecture Notes in Computer Sciences* 5147, 230-244.

Bio Pilot Plant

Borowski A, Kuepper M, **Horn U**, **Knuepfer U**, Zissel G, Haehne K, Luttmann W, Krause S, Virchow JC Jr, Friedrich K (2008) Interleukin-13 acts as an apoptotic effector on lung epithelial cells and induces pro-fibrotic gene expression in lung fibroblasts. *Clinical and Experimental Allergy* 38, 619-628.

Funfak A, Cao J, Wolfbeis OS, **Martin K**, Koehler JM (2008) Monitoring cell cultivation in microfluidic segments by optical pH sensing with a micro flow-through fluorometer using dye-doped polymer particles. *Microchimica Acta* 164, 279-286.

Huang H, He J, Niu X, **Menzel K-D**, Dahse HM, Grabley S, Fiedler P, Sattler I, Hertweck C (2008) Benzopyrenomycin, a cytotoxic bacterial polyketide metabolite with an unprecedented benzo[a]pyrene-type carbacyclic ring system. *Angew Chem Int Ed* 47, 3995-3998.

Li L, Sattler I, Deng Z, Groth I, Walther G, **Menzel K-D**, **Peschel G**, Grabley S, Lin W (2008) A-seco-oleane-type triterpenes from *Phomopsis* sp. (strain HKI0458) isolated from the mangrove plant *Hibiscus tiliaceus*. *Phytochemistry* 69, 511-517.

Li LY, Ding Y, Groth I, **Menzel K-D**, **Peschel G**, Voigt K, Deng ZW, Sattler I, Lin WH (2008) Pyrrole and indole alkaloids from an endophytic *Fusarium incarnatum* (HKI00504) isolated from the mangrove plant *Aegiceras corniculatum*. *J Asian Nat Prod Res* 10, 765-770.

Li Y-Q, Huang X-S, Ishida K, Maier A, Kelter G, Jiang Y, **Peschel G**, **Menzel K-D**, Li M-G, Wen ML, Xu L-H, Grabley S, Fiebig H-H, Jiang Cl, Hertweck C, Sattler I (2008) Plasticity in gilvocarcin-type C-glycoside pathway: discovery and antitumoral evaluation of polycarcin V from *Streptomyces polyformus*. *Org Biomol Chem* 6, 3601-3605.

Niu X, Dahse HM, **Menzel K-D**, Lozach O, Walther G, Meijer L, Grabley S, Sattler I (2008) Butyrolactone I derivatives from *Aspergillus terreus* carrying an unusual sulfate moiety. *J Nat Prod* 71, 689-692.

Ozegowski J-H, **Haertl A**, Bocker T, Schmidt A, Saenger J, **Presselt N**, **Willing K**, **Mueller P-J** (2008) Antiatherosclerotic effect of microbial hyaluronate lyase from group B streptococci. *Die Pharmazie* 63, 601-605.

Richter MEA, Traitcheva N, **Knuepfer U**, Hertweck C (2008) Sequential asymmetric polyketide heterocyclization catalyzed by a single cytochrome P450 monooxygenase (AurH). *Angew Chem Int Ed* 47, 8872-8875.

Schachtschabel D, David A, **Menzel K-D**, Schimek C, Woestemeyer J, Boland W (2008) Cooperative biosynthesis of trisporoids by the (+) and (-) mating types of the zygomycete *Blakeslea trispora*. *ChemBioChem* 9, 2004-2008.

Peer Reviewed Articles 2009
Originalarbeiten 2009

Department Biomolecular Chemistry

Haferburg G, Groth I, Moellmann U, Kothe E, **Sattler I** (2009) Arousing sleeping genes: shifts in secondary metabolism of metal tolerant actinobacteria. *Biometals* 22, 225-234.

Ishida K, Welker M, Christiansen G, Cadel-Six S, Bouchier C, Dittmann E, **Hertweck C**, Tandeau de Marsac N (2009) Plasticity and evolution of aeruginosin biosynthesis in cyanobacteria. *Appl Environ Microbiol* 75, 2017-2026.

Kusebauch B, **Busch B**, **Scherlach K**, Roth M, **Hertweck H** (2009) Polyketide chain branching by an Enzymatic Michael Addition. *Angew Chem Int Ed* 48, 5001-5004.

Lackner G, **Moebius N**, **Scherlach K**, **Partida-Martinez LP**, **Winkler R**, **Schmitt I**, **Hertweck C** (2009) Global distribution and evolution of a roxinogenic *Burkholderia rhizopus symbiosis*. *Appl Environ Microbiol*, 75, 2982-2986.

Meiller TF, Hube B, Schild L, Shirtliff ME, Scheper MA, **Winkler R**, Ton A, Jabra-Rizk MA (2009) A novel immune evasion strategy of *Candida albicans*: proteolytic cleavage of a salivary antimicrobial peptide. *PLoS ONE* 4, e5039.

Melle C, Ernst G, **Winkler R**, Schimmel B, Klussmann JP, Wittekindt C, Guntinas-Lichius O, von Eggeling F (2009) Identification of thioredoxin and epidermal-fatty acid binding protein as up-regulated protein markers in microdissected tumor tissue. *Proteomics* 9, 2193-2201.

Ndejouong Ble S, **Sattler I**, Dahse HM, Kothe E, **Hertweck C** (2009) Isoflavones with unusually modified B-rings and their evaluation as antiproliferative agents. *Bioorg Med Chem Lett* 19, 6473-6476.

Odalo JO, Joseph CC, Nkunya MH, **Sattler I**, **Lange C**, Dahse HM, Moellmann U (2009) Cytotoxic, anti-proliferative and antimicrobial furanoditerpenoids from *Stuhlmania moavi*. *Phytochem* 70, 2047-2052.

Salnikov ES, **Friedrich H**, Li X, Bertani P, Reissmann S, **Hertweck C**, O'Neil JD, Raap J, Bechinger B (2009) Structure and alignment of the membrane-associated peptaibols ampullosporin A and alamethicin by oriented 15N and 31P solid-state NMR spectroscopy. *Biophys J* 96, 86-100.

Schmaler-Ripcke J, Sugareva V, **Gebhardt P**, **Winkler R**, Kniemeyer O, Heinekamp T, Brakhage AA (2009) Production of pyomelanin, a second type of melanin, via the tyrosine degradation pathway in *Aspergillus fumigatus*. *Appl Environ Microbiol* 75, 493-503.

Schroeckh V, **Scherlach K**, Nuetzmann HW, Shelest E, Schmidt-Heck W, **Schuemann J**, Martin K, **Hertweck C**, Brakhage AA (2009) Intimate bacterial-fungal interaction triggers biosynthesis of archetypal polyketides in *Aspergillus nidulans*. *Proc Natl Acad Sci* 106, 14558-14563.

Voedisch M, Albrecht D, Lessing F, Schmidt AD, **Winkler R**, Guthke R, Brakhage AA, Kniemeyer O (2009) Two-dimensional proteome reference maps for the human-pathogenic filamentous fungus *Aspergillus fumigatus*. *Proteomics* 9, 1407-1415.

Wetzel J, **Scheibner O**, Burmester A, Schimek C, Woestemeyer J (2009) 4-Dihydrotrisporin-dehydrogenase, an enzyme of the sex hormone pathway of *Mucor mucedo*: purification, cloning of the corresponding gene, and developmental expression. *Eukaryot Cell* 8, 88-95.

Xu Z, Metsae-Ketelae M, **Hertweck C** (2009) Ketosynthase III as a gateway to engineering the biosynthesis of antitumoral benastatin derivatives. *J Biotechnol* 140, 107-113.

Department Cell and Molecular Biology

Anssour S, Kruegel T, Sharbel TF, **Saluz HP**, Bonaventure G, Baldwin IT (2009) Phenotypic, genetic and genomic consequences of natural and synthetic polyploidization of *Nicotiana attenuata and Nicotiana obtusifolia*. *Ann Bot* 103, 1207-1217.

Mihatsch K, **Nestler M**, **Saluz HP**, Henke A, **Munder T** (2009) Proapoptotic protein siva binds to the muscle protein telethonin in cardiomyocytes during coxsackieviral infection. *Cardiovasc Res* 81, 108-115.

Neumann T, Oelzner P, Freesmeyer M, Hansch A, **Opfermann T**, Hein G, Wolf G (2009) Images in cardiovascular medicine. Diagnosis of large-vessel vasculitis by [18F] fluorodeoxyglucose-positron emission tomography. *Circulation* 119, 338-339.

Schmaler-Ripcke J, Sugareva V, **Gebhardt P**, Winkler R, Kniemeyer O, Heinekamp T, Brakhage AA (2009) Production of pyomelanin, a second type of melanin, via the tyrosine degradation pathway in *Aspergillus fumigatus*. *Appl Environ Microbiol* 75, 493-503.

Department Infection Biology

Altenkaemper M, Bechem B, Perruchon J, Heinrich S, Maedel A, Ortmann R, **Dahse HM**, Freunscht E, Wang Y, Rath J, Stich A, Hitzler M, Chiba P, Lanzer M, Schlitzer M (2009) Antimalarial and antitrypanosomal activity of a series of amide and sulfonamide derivatives of a 2,5-diaminobenzophenone. *Bioorg Med Chem* 17, 7690-7697.

Brissette CA*, **Haupt K***, **Barthel D**, Cooley AE, Bowman A, Welsh E, **Skerka C**, Wallich R, **Zipfel PF**, Kraiczy P, Stevenson B (2009) *Borrelia burgdorferi* infection-associated surface proteins ErpP, ErpA, and ErpC bind human plasminogen. *Infection Immun* 77, 300-306.
* equal contribution.

Buelli S, Abbate M, Morigi M, Moioli D, Zanchi C, Noris M, Zoja C, Pusey C, **Zipfel PF**, Remuzzi G (2009) Loss of factor H-dependent complement regulation mediates proximal tubular cell dysfunction in response to protein overload. *Kidney Intern* 75, 1050-1059.

Doležal R, Waisser K, Petrlikova E, Kuneš J, Kubicová L, Machacek M, Kaustová J, **Dahse HM** (2009) N-benzylsalicylthioamides: Highly active potential antituberculotics. *Arch Pharm (Weinheim)* 342, 113-119.

Gropp K, Schild L, Hube B, **Zipfel PF, Skerka C** (2009) The yeast *Candida albicans* evades the human complement system by secretion of aspartic proteinases. *Mol Immunol* 47, 465-475.

Grosskinsky S, Schott M, Brenner C, Cutler SJ, Kraiczy P, **Zipfel PF**, Simon MM, Wallich R (2009) *Borrelia recurrentis* employs a novel multifunctional surface protein with anti-complement, anti-opsonic and invasive potential to escape innate immunity. *PLoS ONE* 4, e4858.

Hallstroem T, Blom A, **Zipfel PF**, Riesbeck K (2009) Nontypeable *Haemophilus influenzae* protein E binds vitronectin and is important for serum resistance. *J Immunol* 183, 2593-2601.

Heinen S, **Hartmann A**, **Lauer N**, **Wiehl U**, **Dahse HM**, **Schirmer S**, **Gropp K**, **Enghardt T**, Wallich R, **Haelbich S**, **Mihlan M**, Schloetzer-Schrehardt U, **Zipfel PF**, **Skerka C** (2009) Factor H related protein 1 (CFHR-1) inhibits complement C5 convertase activity and terminal complex formation. *Blood* 114, 2439-2447.

Herzberger P, **Siegel C**, Skerka C, Fingerle V, Schulte-Spechtel U, Wilske B, Brade V, **Zipfel PF**, Wallich R, Kraiczy P (2009) A identification and characterization of the factor H and FHL-1 binding complement regulator-acquiring surface protein 1 of the lyme disease spirochete *Borrelia spielmanii* sp. nov. *Int J Med Microbiol* 299, 141-154.

Herzigova P, Klimesova V, Palat K, Kaustova J, **Dahse HM**, Moellmann U (2009) Preparation and *in vitro* evaluation of 4-benylsulfanylpyridine-2-carbohydrazides as potential antituberculosis agents. *Arch Pharm (Weinheim)* 342, 394-404.

Kraiczy P, Hanssen-Huebner C, Kitiratschky V, Brenner C, Besier S, Brade V, Simon MM, **Skerka C**, Roversi P, Lea SM, Stevenson B, Wallich R, Wallich R, **Zipfel PF** (2009) Mutational analyses of the borrelial CRASP-1 surface protein identifies residues relevant for the architecture and binding of the host regulators FHL-1 and factor H. *Int J Med Microbiol* 299, 255-268.

Laube T, Bernet A, **Dahse HM**, Jacobsen ID, Seifert K (2009) Synthesis and pharmacological activity of some sesquiterpene quinones and hydroquinones. *Bioorg Med Chem* 17, 1422-1427.

Licht C, Pluthero FG, Li L, Christensen H, Habbig S, Hoppe B, Geary DF, **Zipfel PF**, Kahr WHA (2009) Platelet-associated complement factor H in normal individuals and patients with atypical HUS. *Blood* 114, 4538-4545.

Luo S, **Poltermann S**, **Kunert A**, Rupp S, **Zipfel PF** (2009) Immune evasion of the human-pathogenic yeast *Candida albicans*: Pra1 is a factor H, FHL-1 and plasminogen binding surface protein. *Mol Immunol* 47, 541-550.

Mache CJ, Acham-Roschitz B, Frémeaux-Bacchi V, Kirschfink M, **Zipfel PF**, Roedl S, Vester U, Ring E (2009) Protective effect of the complement inhibitor eculizumab in an adolescent with atypical Hemolytic Uremic Syndrome. *Clin J Am Soc Nephrol* 4, 1312-1316.

Miethbauer S, Gaube F, Moellmann U, **Dahse HM**, Schmidtke M, Gareis M, Pickhardt M, Liebermann B (2009) Antimicrobial, antiproliferative, cytotoxic, and tau inhibitory activity of rubellins and caeruleoramularin produced by the phytopathogenic fungus *Ramularia collo-cygni* (CAV.) SUTTON & WALLER. *Planta Med* 75, 1523-1525.

Mihlan M, Hebecker M, **Dahse HM**, **Haelbich S**, Huber-Lang M, Dahse R, Zipfel PF, Józsi M (2009) Human complement factor H-related protein 4 binds and recruits native pentameric C-reative protein to necrotic cells. *Mol Immunol* 46, 335-344.

Mihlan M, **Stippa S**, Józsi M, **Zipfel PF** (2009) Monomeric CRP contributes to complement control in fluid phase and on cellular surfaces and increases phagocytosis by recruiting factor H. *Cell Death and Diff* 16, 1630-1640.

Ndejouong Ble S, Sattler I, **Dahse HM**, Kothe E, Hertweck C (2009) Isoflavones with unusual modified B-ring and their evaluation as antiproliferative agents. *Bioorg Med Chem Lett* 19, 6473-6476.

Odalo JO, Joseph CC, Nkunya MHH, Sattler I, Lange C, **Dahse HM** (2009) Cytotoxic, antiproliferative and antimicrobial furanoditerpenoids from *Stuhlmania moavi*. *Phytochemistry* 70, 2047-2052.

Saland JM, Ruggenenti P, Remuzzi G, and the Consensus Study Group Bekassy Z, Bensman A, Trousseau A, Bresin E, Colledan M, Camilla R, Margherita R, Coppo R, Margherita R, Cruzado-Garrit JM, Daina E, Fremeaux-Bacchi V, Goodship TJH, Gridelli B, Hugo C, Karpman D, Jalanko H, LoiratmC, Melgosa M, Mc Kiernan PJ, Noris M, Rodriguez de Cordoba S, Rota G, Miller R, Sanchez-Corral P, **Skerka C**, Tartufari A, Zipfel PF (2009) Liver-kidney transplantation to cure atypical Hemolytic Uremic Syndrome. *J Am Soc Nephrol* 4, 201-206.

Sethi S, Gamez JD, Vrana JA, Theis JD, Bergen III HB, **Zipfel PF**, Dogan A, Smith RJH (2009) Glomeruli of Dense Deposit Disease contain components of the alternative and terminal complement pathway. *Kidney Intern* 75, 952-960.

Skerka C, Józsi M, **Zipfel PF,** Dragon-Durey M-A, Fremeaux-Bacci V (2009) Autoantibodies in Haemolytic Hremic Syndrome (HUS). *Thromb Haemost* 101, 227-232.

Skerka C, Licht C, **Uzonyi B**, Strobel S, **Zipfel PF**, Józsi M (2009) Autoimmune forms of thrombotic micorangiopathy and membranoproliferative glomerulonephritis: indications for a disease spectrum and common pathogenic principles. *Mol Immunol* 46, 2801-2807.

Yunt Z, Reinhardt K, Li A, Engeser M, **Dahse HM**, Guetschow M, Bruhn T, Bringmann G, Piel J (2009) Cleavage of four carbon-carbon bonds during biosynthesis of the griseorhodin a spiroketal pharmacophore. *J Am Chem Soc* 131, 2297-2305.

Department Microbial Pathogenicity Mechanisms

Butler G, Rasmussen MD, Lin MF, Santos MAS, Sakthikumar S, Munro CA, Rheinbay E, Grabherr M, Forche A, Reedy JL, Agrafioti I, Arnaud MB, Bates S, Brown AJP, **Brunke S**, Costanzo MC, Fitzpatrick DA, de Groot PWJ, Harris D, Hoyer LL, **Hube B**, Klis FM, Kodira C, Lennard N, Logue ME, **Martin R**, Neiman AM, Nikolaou E, Quail MA, Quinn J, Santos MC, Schmitzberger FF, Sherlock G, Shah P, Silverstein K, Skrzypek MS, Soll D, Staggs R, Stansfield I, Stumpf MPH, Sudbery PE, Thyagarajan S, Zeng Q, Berman J, Berriman M, Heitman J, Gow NAR, Lorenz MC, Birren BW, Kellis M, Cuomo CA (2009) Evolution of pathogenicity and sexual reproduction in eight *Candida* genomes. *Nature* 459, 657-662.

Dalle F, **Waechtler B**, Coralie L'O, Holland G, Bannert N, **Wilson D**, Labruère C, Bonnin A, **Hube B** (2009) Cellular interactions of *Candida albicans* with human oral epithelial cells and enterocytes. *Cellular Microbiology* 12, 248-271.

Domin N, **Wilson D**, Brock M (2009) Methylcitrate cycle activation during adaptation of *Fusarium solani* and *Fusarium verticillioides* to propionyl-CoA generating carbon sources. *Microbiol* 155, 3903-3912.

Gropp K, **Schild L**, Schindler S, Hube B, Zipfel PF, Skerka C (2009) The yeast *Candida albicans* evades the human complement system by secretion of aspartic proteinases. *Molecular Immunology* 47, 465-475.

Hornbach A, **Heyken A**, **Schild L**, **Hube B**, Loeffler J, Kurzai O (2009) Secreted aspartic protease Sap9 modulates the interaction of *Candida albicans* with human neutrophils. *Infect Immun* 77, 5216-5224.

Hort W, Lang S, **Brunke S**, Mayser P, **Hube B** (2009) Analysis of differ-

entially expressed genes associated with tryptophan-dependent pigment synthesis in *M. furfur* by cDNA subtraction technology. *Med Mycol* 47, 248-258.

Laube T, Bernet A, Dahse HM, **Jacobsen ID**, Seifert K (2009) Synthesis and pharmacological activities of some sesquiterpene quinones and hydroquinones. *Bioorg Med Chem* 17, 1422-1427.

Meiller TF, **Hube B**, **Schild L**, Shirtliff ME, Scheper MA, Winkler R, Ton A, Jabra-Rizk MA (2009) A novel immune evasion strategy of *Candida albicans*: proteolytic cleavage of a salivary antimicrobial peptide. *PLoS ONE* 4, e5039.

Voelksch B, Thon S, **Jacobsen ID**, Gube M (2009) Polyphasic study of plant- and clinic-associated *Pantoea agglomerans* strains reveals indistinguishable virulence potential. *Infect Genet* 9, 1381-1391.

Department Molecular and Applied Microbiology

Aimanianda V, Bayry J, Bozza S, **Kniemeyer O**, Perruccio K, Elluru SR, Clavaud C, Paris S, **Brakhage AA**, Kaveri SV, Romani L, Latgé JP (2009) Surface hydrophobin prevents immune recognition of airborne fungal spores. *Nature* 460, 1117-1121.

Guenther S, **Groth I**, Schierack P, Grabley S, Munder T (2009) Direct detection of *Kitasatospora* species with a chaperone oligonucleotide microarray method lacking PCR amplification. *J Basic Microbiol* 48, 315-318.

Haferburg G, **Groth I**, **Moellmann U**, Kothe E, Sattler I (2009) Arousing sleeping genes: shifts in secondary metabolism of metal tolerant actinobacteria under conditions of heavy metal stress. *Biometals* 22, 225-234.

Hauburger A, von Einem S, Schwaerzer GK, Buttstedt A, Zebisch M, Schraeml M, **Hortschansky P**, Knaus P, Schwarz E (2009) The proform of BMP-2 interferes with BMP-2 signaling by competing with BMP-2 for IA receptor binding. *FEBS J* 276, 6386-6398.

Herzigova P, Klimesova V, Palat K, Kaustova J, Dahse HM, **Moellmann U** (2009) Preparation and *in vitro* evaluation of 4-benzylsulfanylpyridine-2-carbohydrazides as potential antituberculosis agents. *Arch Pharm Chem Life Sci* 342, 394-404.

Lokaj K, Meierjohann S, Schuetz C, **Teutschbein J**, Schartl M, Sickmann A (2009) Quantitative differential proteome analysis in an animal model for human melanoma. *J Proteome Res* 8,1818-1827.

Makarov V*, Manina G*, Mikusova K*, **Moellmann U***, Ryabova O, Saint-Joanis B, Dhar N, Pasca MR, Buroni S, Lucarelli AP, Milano A, De Rossi E, Belanova M, Bobovska A, Dianiskova P, Kordulakova J, Sala C, Fullam E, Schneider P, McKinney JD, Brodin P, Christophe T, Waddell S, Butcher P, Albrethsen J, Rosenkrands I, Brosch R, Nandi V, Bharath S, Gaonkar S, Shandil RK, Balasubramanian V, Balganesh T, Tyagi S, Grosset J, Riccardi G, Cole ST (2009) Benzothiazinones kill *Mycobacterium tuberculosis* by blocking arabinan synthesis. *Science* 324, 801-804. * equal contribution.

Meinhardt J, Sachse C, **Hortschansky P**, Grigorieff N, Faendrich M (2009) Abeta(1-40) fibril polymorphism implies diverse interaction patterns in amyloid fibrils. *J Mol Biol* 386, 869-877.

Miethbauer S, Gaube F, **Moellmann U**, Dahse HM, Schmidtke M, Gareis M, Pickhardt M, Liebermann B (2009) Antimicrobial, antiproliferative, cytotoxic, and tau inhibitory activity of rubellins and caeruleoramularin produced by the phytopathogenic fungus *Ramularia collo-cygni*. *Planta Medica* 75, 1523-1525.

Miller MJ, Zhu H, Xu Y, Wu C, Walz AJ, Vergne A, Roosenberg JM, Moraski G, Minnick AA, McKee-Dolence J, Hu J, Fennel K, Dolence EK, Dong L, Franzblau S, Malouin F, **Moellmann U** (2009) Utilization of microbial iron assimilation processes for the development of new antibiotics and inspiration for the design of new anticancer agents. *Biometals* 22, 61–75.

Moellmann U, Heinisch L, Bauernfeind A, Koehler T, Ankel-Fuchs D (2009) Siderophores as drug delivery agents: application of the ''Trojan Horse'' strategy. *Biometals* 22, 615–624.

Rispail N, Soanes DM, Ant C, Czajkowski R, Gruenler A, Huguet R, Perez-Nadales E, Poli A, Sartorel E, **Valiante V**, Yang M, Beffa R, **Brakhage AA**, Gow NA, Kahmann R, Lebrun MH, Lenasi H, Perez-Martin J, Talbot NJ, Wendland J, Di Pietro A (2009) Comparative genomics of MAP kinase and calcium-calcineurin signalling components in plant and human-pathogenic fungi. *Fungal Genet Biol* 46, 287-298.
Sammer UF, Voelksch B, **Moellmann U**, Schmidtke M, Spiteller P, Spiteller M, Spiteller D (2009) 2-Amino-3-(oxirane-2,3-dicarboxamido)-propanoyl-valine, an effective peptide antibiotic from the epiphyte *Pantoea agglomerans* 48b/90. *Appl Environ Microbiol* 75, 7710-7717.

San Miguel B, Ghayor C, Ehrbar M, Jung RE, Zwahlen R, **Hortschansky P**, Schmoekel H, Weber F (2009) N-methyl pyrrolidone as a potent bone morphogenetic protein enhancer for bone tissue regeneration. *Tissue Eng Part A* 15, 2955-2963.

Schaefer J, Trautmann C, Dill I, Fischer G, Gabrio T, **Groth I**, Jaeckel U, Lorenz W, Martin K, Miljanic T, Szewzyk R, Weidner U, Kaempfer P (2009) Vorkommen von Actinomyceten in Innenräumen. *Gefahrstoffe – Reinhaltung der Luft* 9/2009, 335-341.

Schmaler-Ripcke J, **Sugareva V**, Gebhardt P, Winkler R, **Kniemeyer O**, **Heinekamp T**, **Brakhage AA** (2009) Production of pyomelanin, a second type of melanin, via the tyrosine degradation pathway in *Aspergillus fumigatus*. *Appl Environ Microbiol* 75, 493-503.

Schroeckh V, Scherlach K, **Nuetzmann HW**, Shelest E, Schmidt-Heck W, Schuemann J, Martin K, Hertweck C, **Brakhage AA** (2009) Intimate bacterial-fungal interaction triggers biosynthesis of archetypal polyketides in *Aspergillus nidulans*. *Proc Natl Acad Sci USA* 106, 14558-14563.

Sproete P, **Brakhage AA**, Hynes MJ (2009) Contribution of peroxisomes to penicillin biosynthesis in *Aspergillus nidulans*. *Eukaryot Cell* 8, 421-423.

Teutschbein J, Schartl M, Meierjohann S (2009) Interaction of *Xiphophorus* and murine Fyn with focal adhesion kinase. *Comp Biochem Physiol C Toxicol Pharmacol* 149, 168-174.

Teutschbein J, Schumann G, **Moellmann U**, Grabley S, Cole ST, Munder T (2009) A protein linkage map of the ESAT-6 secretion system (ESX-1) of *Mycobacterium tuberculosis*. *Microbiol Res* 164, 253-259.

Valiante V, **Jain R**, **Heinekamp T**, **Brakhage AA** (2009) The MpkA MAP kinase module regulates cell wall integrity signaling and pyomelanin formation in *Aspergillus fumigatus*. *Fungal Gene Biol* 46, 909-918.

Voedisch M, Albrecht D, **Lessing F, Schmidt A**, Winkler R, Guthke R, **Brakhage AA**, **Kniemeyer O** (2009) Two-dimensional proteome reference maps for the human-pathogenic filamentous fungus *Aspergillus fumigatus*. *Proteomics* 9, 1407-1415.

Wencewicz TA, **Moellmann U**, Long TE, Miller MJ (2009) Is drug release necessary for antimicrobial activity of siderophore-drug conjugates? Syntheses and biological studies of the naturally occurring salmycin ''Trojan Horse'' antibiotics and synthetic desferridanoxamine-antibiotic conjugates. *Biometals* 22, 633-648.

Yang B, Miller PA, **Moellmann U**, Miller MJ (2009) Syntheses and biological activity studies of novel sterol analogs from nitroso Diels-Alder reactions of ergosterol. *Org Lett* 11, 2828-2831.

Junior Research Group Cellular Immunobiology

Mihlan M, **Hebecker M**, Dahse HM, Haelbich S, Huber-Lang M, Dahse R, Zipfel PF, **Józsi M** (2009) Human complement factor H-related protein 4 binds and recruits native pentameric C-reactive protein to necrotic cells. *Mol Immunol* 46, 335-344.

Mihlan M, Stippa S, **Józsi M**, Zipfel PF (2009) Monomeric CRP contributes to complement control in fluid phase and on cellular surfaces and increases phagocytosis by recruiting factor H. *Cell Death Differ* 16, 1630-1640.

Junior Research Group Microbial Biochemistry and Physiology

Domin N, Wilson D, **Brock M** (2009) Methylcitrate cycle activation during adaptation of *Fusarium solani* and *Fusarium verticillioides* to propionyl-CoA generating carbon sources. *Microbiology-SGM* 155, 3903-3912.

Fleck CB, **Brock M** (2009) Re-characterisation of *Saccharomyces cerevisiae* Ach1p: fungal CoA-transferases are involved in acetic acid detoxification. *Fungal Genet Biol* 46, 473-485.

Junior Research Group Fundamental Molecular Biology of Pathogenic Fungi

Rennemeier C, Frambach T, **Hennicke F**, Dietl J, **Staib P** (2009) Microbial quorum sensing molecules induce acrosome loss and cell death in human spermatozoa. *Infect Immun* 77, 4990-4997.

Zaugg C, Monod M, Weber J, Harshman K, Pradervand S, Thomas J,

Bueno M, Giddey K, **Staib P** (2009) Gene expression profiling in the human-pathogenic dermatophyte *Trichophyton rubrum* during growth on proteins. *Eukaryot Cell* 8, 241-250.

Junior Research Group Secondary Metabolism of Predatory Bacteria

Nett M, Gulder TAM, Kale AJ, Hughes CC, Moore BS (2009) Function-oriented biosynthesis of β-lactone proteasome inhibitors in *Salinispora tropica*. *J Med Chem* 52, 6163-6167.

Penn K, Jenkins C, **Nett M**, Udwary DW, Gontang EA, McGlinchey RP, Foster B, Lapidus A, Podell S, Allen EE, Moore BS, Jensen PR (2009) Genomic islands link secondary metabolism to functional adaptation in marine actinobacteria. *ISME J* 3, 1193-1203.

Schneider P, Jacobs JM, Neres J, Aldrich CC, Allen C, **Nett M**, Hoffmeister D (2009) The global virulence regulators VsrAD and PhcA control secondary metabolism in the plant pathogen *Ralstonia solanacearum*. *ChemBioChem* 10, 2730-2732.

Research Group Fungal Septomics

Brissette CA, **Haupt K**, Barthel D, Cooley AE, Bowman A, Skerka C, Wallich R, Zipfel PF, Kraiczy P, Stevenson B (2009) *Borrelia burgdorferi* infection-associated surface proteins ErpP, ErpA, and ErpC bind human plasminogen. *Infect Immun* 77, 300-306.

Butler G, Rasmussen MD, Lin MF, Santos MA, Sakthikumar S, Munro CA, Rheinbay E, Grabherr M, Forche A, Reedy JL, Agrafioti I, Arnaud MB, Bates S, Brown AJ, Brunke S, Costanzo MC, Fitzpatrick DA, de Groot PW, Harris D, Hoyer LL, Hube B, Klis FM, Kodira C, Lennard N, Logue ME, **Martin R**, Neiman AM, Nikolaou E, Quail MA, Quinn J, Santos MC, Schmitzberger FF, Sherlock G, Shah P, Silverstein KA, Skrzypek MS, Soll D, Staggs R, Stansfield I, Stumpf MP, Sudbery PE, Srikantha T, Zeng Q, Berman J, Berriman M, Heitman J, Gow NA, Lorenz MC, Birren BW, Kellis M, Cuomo CA (2009) Evolution of pathogenicity and sexual reproduction in eight *Candida* genomes. *Nature* 459, 657-662.

Hornbach A, Heyken A, Schild L, Hube B, Loeffler J, **Kurzai O** (2009) The glycosylphosphatidylinositol-anchored protease Sap9 modulates the interaction of *Candida albicans* with human neutrophils. *Infect Immun* 77, 5216-5224.

Ok M, Latgé JP, Baeuerlein C, Ebel F, Mezger M, Topp M, **Kurzai O**, Kilian D, Kapp M, Grigoleit GU, Sennefelder H, Arroyo J, Einsele H, Loeffler J (2009) Immune responses of human immature dendritic cells can be modulated by the recombinant *Aspergillus fumigatus* antigen Aspf1. *Clin Vaccine Immunol* 16, 1485-1492.

Schielke S, Huebner C, Spatz C, Naegele V, Ackermann N, Frosch M, **Kurzai O**, Schubert-Unkmeir A (2009) Expression of the meningococcal adhesin NadA is controlled by a transcriptional regulator of the MarR family. *Mol Microbiol* 72, 1054-1067.

Research Group Pharmaceutical Biology

Misiek M, Williams J, Schmich K, Huettel W, Merfort I, Salomon C, Aldrich CA, **Hoffmeister D** (2009) Structure and Cytotoxicity of arnamial and related fungal sesquiterpene aryl esters. *J Nat Prod* 72, 1888-1891.

Schneider P, Jacobs J, Neres J, Aldrich CA, Allen CZ, Nett M, **Hoffmeister D** (2009) The global virulence regulators VsrAD and PhcA control secondary metabolism in the plant pathogen *Ralstonia solanacearum*. *ChemBiochem* 10, 2730-2732.

Research Group Systems Biology / Bioinformatics

Acikgoez A, Karim N, Giri S, **Schmidt-Heck W**, Bader A (2009) Two compartment model of diazepam biotransformation in an organotypical culture of primary human hepatocytes. *Toxicology and Applied Pharmacology* 234, 179-191.

Friedel M, **Nikolajewa S**, Suehnel S, Wilhelm T (2009) DiProDB: a database for dinucleotide properties. *Nucleic Acids Res* 37 (Database issue), D37-D40.

Friedel M, **Nikolajewa S**, Suehnel S, Wilhelm T (2009) DiProGB: the dinucleotide properties genome browser. *Bioinformatics* 25, 2603-2604.

Graebner R, Loetzer K, Doepping S, Hildner M, **Radke D**, Beer M, Spanbroek R, Lippert B, Reardon CA, Getz GS, Fu YX, Hehlgans T, Mebius RE, van der Wall M, Kruspe D, Englert C, Lovas A, Hu D, Randolph GJ, Weih F, Habenicht AJ (2009) Lymphotoxin beta receptor signaling promotes tertiary lymphoid organogenesis in the *aorta adventitia* of aged ApoE-/- mice. *J Exp Med* 206, 233-248.

Hecker M, Goertsches RH, Engelmann R, Thiesen HJ, **Guthke R** (2009) Integrative modeling of transcriptional regulation in response to antirheumatic therapy. *BMC Bioinformatics* 10, 262.

Kienbaum M, Koy C, Montgomery HV, Drynda S, Lorenz P, Illges H, Tanaka K, Kekow J, **Guthke R**, Thiesen HJ, Glocker MO (2009) Mass spectrometric characterization of apheresis samples from rheumatoid arthritis patients for the improvement of immunoadsorption therapy - a pilot study. *Proteomics - Clin Appl* 3, 797-809.

Linde J, Olsson B, Lubovac Z. (2009) Network properties for ranking predicted miRNA targets in breast cancer. *Adv Bioinformatics* 2009, 182689.

Schroeckh V, Scherlach K, Nuetzmann H-W, **Shelest E**, **Schmidt-Heck W**, Schuemann J, Martin K, Hertweck C, Brakhage AA (2009) Intimate bacterial-fungal interaction triggers biosynthesis of archetypal polyketides in *Aspergillus nidulans*. *PNAS USA* 106, 14558-14563.

Sinha R, **Nikolajewa S**, Szafranski K, Hiller M, Jahn N, Huse K, Platzer M, Backofen R (2009) Accurate prediction of NAGNAG alternative splicing. *Nucleic Acids Res* 37, 3569-3579.

Voedisch M, **Albrecht D**, Lessing F, Schmidt AD, Winkler R, **Guthke R**, Brakhage AA, Kniemeyer O (2009) Two-dimensional proteome reference maps for the human-pathogenic filamentous fungus *Aspergillus fumigatus*. *Proteomics* 9, 1407-1415.

Wollbold J, Huber R, Pohlers D, Koczan D, **Guthke R**, Kinne RW, Gausmann U (2009) Adapted boolean network models for extracellular matrix formation. *BMC Systems Biology* 3, 77.

Zellmer S, **Sickinger S**, **Schmidt-Heck W**, **Guthke R**, Gebhardt R (2009) Heterogeneous expression of suppressor of cytokine signaling 2 (SOCS-2) in liver tissue. *J Anat* 215, 176-183.

Bio Pilot Plant

Funfak A, Hartung R, Cao J, **Martin K**, Wiesmueller K-H, Wolfbeis OS, Koehler JM (2009) Highly resolved dose-response functions for drug-modulated bacteria cultivation obtained by fluorometric and photometric flow-through sensing in microsegmented flow. *Sensors and Actuators B Chemical* 142, 66-72.

Kaempfer P, **Martin K**, Schaefer J, Schumann P (2009) *Kytococcus aerolatus* sp. nov., isolated from indoor air in a room colonized with moulds. *System Appl Microbiol* 32, 301-305.

Kawe M, **Horn U**, Plueckthun A (2009) Facile promoter deletion in Escherichia coli in response to leaky expression of very robust and benign proteins from common expression vectors. *Microbial Cell Factories* 8, 8.

Kensy F, **Zang E**, Faulhammer C, Tan RK, Buechs J (2009) Validation of a high-throughput fermentation system based on online monitoring of biomass and fluorescence in continuously shaken microtiter plates. *Microb Cell Fact* 8, 31.

Kusebauch B, Busch B, Scherlach K, **Roth M**, Hertweck C (2009) Polyketide chain branching by an enzymatic Michael addition. *Angew Chem Int Ed* 48, 5001-5004.

Schaefer J, Trautmann C, Dill I, Fischer G, Gabrio T, Groth I, Jaeckel U, Lorenz W, **Martin K**, Miljanic T, Szewzyk R, Weidner U, Kaempfer P (2009) Vorkommen von Actinomyceten in Innenräumen. *Gefahrstoffe – Reinhaltung der Luft* 9/2009, 335-341.

Schroeckh V, Scherlach K, Nuetzmann HW, Shelest E, Schmidt-Heck W, Schuemann J, **Martin K**, Hertweck C, Brakhage AA (2009) Intimate bacterial-fungal interaction triggers biosynthesis of archetypal polyketides in *Aspergillus nidulans*. *Proc Natl Acad Sci USA* 106, 14558-14563.

Reviews, Monographs, Book chapters 2008/2009
Übersichtsarbeiten, Monographien, Beiträge zu Sammelwerken 2008/2009

Department Biomolecular Chemistry

Brakhage AA, Bergmann S, **Schuemann J**, **Scherlach K**, Schroeckh V, **Hertweck C** (2009) Fungal genome mining and activation of silent gene clusters. In: Anke T, Weber D (Eds.) The Mycota XV, *Physiology and Genetics*, 297-303.

Busch B, **Hertweck C** (2009) Evolution of metabolic diversity in polyketide-derived pyrones. *Phytochem* 70, 1833-1840.

Hertweck C (2009) Hidden biosynthetic treasures brought to light. *Nat Chem Biol* 5, 450-452.

Hertweck C (2009) The biosynthetic logic of polyketide diversity. *Angew Chem Int Ed* 48, 4688-4716.

Lackner G, **Partida-Martinez LP**, **Hertweck C** (2009) Endofungal bacteria as producers of mycotoxins. *Trends Microbiol* 17, 570-576.

Moebius N, **Hertweck C** (2009) Fungal phytotoxins as mediators of virulence. *Curr Opin Plant Biol* 12, 390-398.

Osada H, **Hertweck C** (2009) Exploring the chemical space of microbial natural products. *Curr Opin Chem Biol* 13, 133-134.

Scherlach K, **Hertweck C** (2009) Triggering cryptic natural product biosynthesis in microorganisms. *Org Biomol Chem* 7, 1753-1760.

Schuemann J, **Hertweck C** (2009) Biosynthesis of fungal polyketides. In: Anke T, Weber D (Eds.) The Mycota XV, *Physiology and Genetics*, 331-351.

Department Infection Biology

Brakhage AA, **Zipfel PF** (Eds.) (2008) The Mycota, Springer.

Józsi M, **Zipfel PF** (2008) Factor H-related proteins and complement-associated diseases. *Trends in Immunology* 8, 380-387.

Skerka C, **Zipfel PF** (2008) Complement factor H related proteins in immune diseases. *Vaccine* 26, I9-14.

Zipfel PF (2008) Immune Evasion der human-pathogenen Hefe *Candida albicans. Die Naturheilkunde* 4, 11-13.

Zipfel PF, **Schindler S**, **Reuter M**, **Gropp K**, **Skerka C** (2008) *Candida* and complement. In: Brakhage AA, Zipfel PF (Eds.) The Mycota, Springer.

Zipfel PF, **Skerka C** (2008) Complement: the alternative pathway. In: Wiley J & Sons (Eds.) Encyclopedia of Life Sciences (ELS), Ltd: Chichester. DOI: 10.1002/9780470015902.a0000509.pub2.

Brakhage AA, **Zipfel PF** (2009) Chapter 13: Overview about fungal pathogens in immunology of infectious diseases. In: Kaufmann SHE, Rouse B, Sacks D (Eds.) American Society of Microbiology, ASM Book. in press.

Eberhardt HU, **Uzonyi B**, **Haelbich S**, **Zipfel PF**, **Skerka C** (2009) Complement factor H-related protein 2 (CFHR2) is a C3b and heparin binding protein. *Medimond International Proceedings ECI Berlin*, 5-9.

Reuter M, **Zipfel PF** (2009) Complement activation products C3a and C4a as endogenous antimicrobial peptides. *Int J Peptid Res Therap* 15, 87-95.

Schroeder H, Skelly P, **Zipfel PF**, Losson B, Vanderplasschen A (2009) Subversion of complement by hematophagous parasites. *Develop Comp Immunol* 33, 5-13.

Skerka C, Józsi M, **Zipfel PF**, Dragon-Durey M, Fremeaux-Bacchi V (2009) Autoantibodies in Hemolytic Uremic Syndrome (HUS). *Thrombosis and Haemostasis* 101, 227-232.

Zipfel PF (2009) Complement and immune defense: from innate immunity to human diseases. *Immunol Letters* 126, 1-7.

Zipfel PF (2009) Thrombotic microangiopathies as a prime example for translational medicine. *Thrombosis and Haemostasis* 101, 225-226.

Zipfel PF, **Luo S**, **Schindler S** (2009) Immunbiologie von humanpathogenen Hefen und Schimmelpilzen. *Wohnmedizin* 1, 9-14.

Zipfel PF, **Skerka C** (2009) Complement regulators and inhibitory proteins. *Nature Reviews Immunology* 10, 729-740.

Zipfel PF, Smith RJH, **Skerka C** (2009) Complement in focus: factor I and factor H deficiency in renal diseases: similar defects in fluid phase have a different outcome at the surface of the glomerular basement membrane. *Editorial Nephrol Dial Transplantation* 24, 385-387.

Department Microbial Pathogenicity Mechanisms

Hube B (2008) *Candida*, Proteasen und Gene. *Mycoses* 51, 22-25.

Hube B (2008) Forschung an human-pathogenen Pilzen bündeln und koordinieren. The first joint meeting of research consortia on fungal pathogens in Europe 2007, 12.-15. September 2007, Gosau (Österreich), *Biospektrum* 1, 102. Report on FunNet 2007.

Schaller M, Zakikhany K, Weindl G, Borelli C, Korting HC, **Hube B** (2008) *In vitro*-Modelle auf der Basis von rekonstituierter humaner Haut/Schleimhaut zur Charakterisierung der Interaktion zwischen *C. albicans* und Wirtszellen. *Der Hautarzt* 59, 964-970.

Thewes S, **Hube B** (2008) Untersuchungen zur Invasivität von *Candida albicans. Epidemiologisches Bulletin* 29, 230-231.

Zakikhany K, Thewes S, Wilson D, Martin R, **Albrecht A**, **Hube B** (2008) From attachment to invasion: Infection associated genes of *Candida albicans. Nippon Ishinkin Gakkai Zasshi* 49, 245-251.

Hube B (2009) Fungal adaptation to the host environment. *Current Opinion in Microbiology* 12, 1-3.

Almeida RS, **Wilson D**, **Hube B** (2009) *Candida albicans* iron acquisition within the host. *FEMS Yeast Research* 9, 1000-1012.

Wilson D, Thewes S, Zakikhany K, Fradin C, **Albrecht A**, **Almeida R**, **Brunke S**, **Grosse K**, **Martin R**, **Mayer F**, **Leonhardt I**, **Schild L**, **Seider K**, **Skibbe M**, **Slesiona S**, **Waechtler B**, **Jacobsen I**, **Hube B** (2009) Identifying infection-associated genes of *Candida albicans* in the post genomic era. *FEMS Yeast Research* 9, 688-700.

Naglik JR, **Hube B** (2009) Chapter 9: secreted *Candida* proteins: pathogenicity and host immunity. In: Bignell E, Ashbee R (Eds.) Handbook of pathogenic yeasts, 97-120.

Schaller M, Weindl G, **Hube B** (2009) Introduction: host responses. *Methods Mol Biol* 470, 291-292.

Department Molecular and Applied Microbiology

Albrecht D, **Guthke R**, **Kniemeyer O**, **Brakhage AA** (2008) Systems biology of human-pathogenic fungi (chap. XXIV). In: Daskalaki A (Ed.) Handbook of research on systems biology applications in medicine, Idea Group Inc, 400-418.

Albrecht D, **Kniemeyer O**, **Guthke R**, **Brakhage AA** (2008) Normalisation of 2D DIGE data – on the way to a standard operating procedure. *BIRD08 Schriftenreihe Informatik* 26, 55-64.

Kniemeyer O, **Brakhage AA** (2008) Proteomics and its application to the human-pathogenic fungi *Aspergillus fumigatus* and *Candida albicans*. In: Brakhage AA, Zipfel PF (Eds.) The Mycota VI-Human and Animal Relationships, Springer, Berlin.

Brakhage AA, **Bergmann S**, Schuemann J, Scherlach K, **Schroeckh V**, Hertweck C (2009) Fungal genome mining and activation of silent gene clusters. In: Anke T, Weber D (Eds.) The Mycota XV, Physiology and Genetics.

Brakhage AA, Gierl A, Hartmann T, Strack D (2009) Evolution of metabolic diversity. *Phytochemistry* 70, 1619-1620.

Brakhage AA, **Thoen M**, **Sproete P**, **Scharf DH**, **Al-Abdallah Q**, **Wolke SM**, **Hortschansky P** (2009) Aspects on evolution of fungal beta-lactam biosynthesis gene clusters and recruitment of trans-acting factors. *Phytochemistry* 70, 1801-1811.

Kniemeyer O, **Lessing F**, **Brakhage AA** (2009) Proteome analysis for pathogenicity and new diagnostic markers for *Aspergillus fumigatus*. *Med Mycol* 47, 248-254.

Schaefer J, Trautmann C, Dill I, Fischer G, Gabrio T, **Groth I**, Jaeckel U, Lorenz W, Martin K, Miljanic T, Szewzyk R, Weidner U, Kaempfer P (2009) Vorkommen von Actinomyceten in Innenräumen. In: Gefahrstoffe-Reinhaltung der Luft 9, Springer-VDI-Verlag, 335-341.

Junior Research Group Cellular Immunobiology (Dr. Mihály Józsi)

Józsi M, Zipfel PF (2008) Factor H family proteins and human diseases. *Trends in Immunology* 29, 380-387.

Skerka C, **Józsi M**, Zipfel PF, Dragon-Durey MA, Fremeaux-Bacchi V (2009) Autoantibodies in Hemolytic Uraemic Syndrome (HUS). *Thromb Haemost* 101, 227-232.

Skerka C, Licht C, Mengel M, Uzonyi B, **Strobel S**, Zipfel PF, **Józsi M** (2009) Autoimmune forms of thrombotic microangiopathy and membranoproliferative glomerulonephritis: indications for a disease spectrum and common pathogenic principles. *Mol Immunol* 46, 2801-2807.

Junior Research Group Microbial Biochemistry and Physiology

Brock M (2008) Physiology and metabolic requirements of pathogenic fungi. In: Esser K (Ed.) The Mycota IV-A Comprehensive Trea-

tise on Fungi as Experimental System for Basic and Applied Research, Vol. VI, Springer, Heidelberg-Berlin, 63-82.

Brock M (2009) Crystal ball 2009: back to the roots. *Microbiol Biotechnol* 2, 132-133.

Brock M (2009) Fungal metabolism in host niches. *Curr Opin Microbiol* 12, 371-376.

Brock M (2009) Role of cellular control of propionyl-CoA levels for microbial pathogenesis. *Handbook of Hydrocarbon and Lipid Microbiology*. in press.

Junior Research Group Secondary Metabolism of Predatory Bacteria

Nett M, Ikeda H, Moore BS (2009) Genomic basis for natural product biosynthetic diversity in the actinomycetes. *Nat Prod Rep* 26, 1362-1384.

Nett M, Moore BS (2009) Exploration and engineering of biosynthetic pathways in the marine actinomycete *Salinispora tropica*. *Pure Appl Chem* 81, 1075-1084.

Junior Research Fungal Septomics

Kurzai O (2009) Nosokomiale Meningitis. In: Frosch M (Ed.) Akute bakterielle Meningitis, Unimed Verlag AG Bremen, 2. Auflage 2009.

Schmitt C, Villwock A, **Kurzai O** (2009) Recognition of meningococcal molecular patterns by innate immune receptors. *Int J Med Microbiol* 299, 9-20.

Wilson D, Thewes S, Zakikhany K, Fradin C, Albrecht A, Almeida R, Brunke S, Grosse K, **Martin R**, Mayer F, **Leonhardt I**, Schild L, Seider K, Skibbe M, Slesiona S, Waechtler B, Jacobsen I, Hube B (2009) Identifying infection-associated genes of Candida albicans in the postgenomic era. *FEMS Yeast Res* 9, 688-700.

Zakikhany K, Thewes S, Wilson D, **Martin R**, Albrecht A, Hube B (2008) From attachment to invasion: infection associated genes of *Candida albicans*. *Nippon Ishinkin Gakkai Zasshi* 49, 245-251.

Research Group Pharmaceutical Biology

Hoffmeister D, Bechthold A (2009) Giftpflanzen, Pilzgifte. In: Aktories K, Foerstermann U, Hofmann F, Starke K (Eds.) Repetitorium Pharmakologie und Toxikologie, 2. Auflage, Elsevier, 447-451.

Hoffmeister D, Bechthold A (2009) Kapitel 37.12 und 37.13 (Giftpflanzen, Pilzgifte). In: Aktories K, Foerstermann U, Hofmann F, Starke K (Eds.) Pharmakologie und Toxikologie, 10. Auflage, Elsevier, 1098-1117.

Schneider P, Misiek M, **Hoffmeister D** (2008) In vivo and in vitro production options for fungal natural products. *Mol Pharm* 5, 234-242.

Research Group Systems Biology / Bioinformatics

Hecker M, **Lambeck S**, Toepfer S, van Someren E, **Guthke R** (2009) Gene regulatory network inference - data integration in dynamic models - a review. *BioSystems* 96, 86-103.

Moeller U (2009) Resampling methods for unsupervised learning from sample data. In: Mellouk A, Chebira A (Eds.) Machine Learning http://www.intechweb.org, in-tech review.

Memberships in Editorial Boards 2008/2009
Mitgliedschaften in Editorial Boards 2008/2007

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Applied Environmental Microbiology
Archives of Microbiology
Current Genetics
Applied Microbiology and Biotechnology

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Forschungszentrum Borstel, Leibniz-Zentrum für Medizin und Biowissenschaften
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18.03.2008
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Karl-Heinz Wiesmüller
EMC microcollections GmbH, Tübingen
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15.04.2008
Host: Roth M

Oliver Kurzai
Julius-Maximilians-Universität Würzburg
Pathogen or commensal? -recognition of *C. albicans* by neutrophils
29.04.2008
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Sucharit Bhakdi
Johannes Gutenberg-Universität Mainz
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08.05.2008
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Hildgund Schrempf
Universität Osnabrück
Protein-vermittelte Interaktionen zwischen Streptomyceten und Pilzen
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Host: Brakhage AA, Horn U

Jochen Süss
Friedrich-Loeffler-Institut – Bundesforschungsinstitut für Tiergesundheit
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10.06.2008
Host: Zipfel PF, Stelzner A, Brakhage AA

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Rheinische Friedrich-Wilhelms-Universität Bonn
Die tragbare Chemiefabrik: Tierwirkstoffe aus bakteriellen Symbionten
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Lund University, Malmö, Sweden
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RIKEN Advanced Science Institute, Wako, Japan
Chemical biology on osteoclast

inhibitors isolated from micro-organisms
05.08.2008
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Klaus Benndorf
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Activation of CNG and HCN channels with cyclic nucleotides allows to directly relate ligand binding conformational change in a receptor protein
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Klaus Höffken
Universitätsklinikum Jena
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18.11.2008
Host: Zipfel PF, Stelzner A, Brakhage AA

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Helmholtz-Zentrum Dresden-Rossendorf
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11.02.2009
Host: Brakhage AA

James F. Beck
Universitätsklinikum Jena
Angeborene Immundefekte
03.03.2009
Host: Brakhage AA, Zipfel PF, Stelzner A

Ernst Th. Rietschel
Präsident der Leibniz-Gemeinschaft
Unsterbliche Musik und tödliche Blutvergiftung – Vom Sepsistod berühmter Komponisten
23.04.2009
Host: Brakhage AA

Christoph Binder
Medizinische Universität Wien, Austria
Oxidation-specific epitopes are important targets of innate immunity
12.05.2009
Host: Zipfel PF

Matthias Dürst
Universitätsklinikum Jena
Interventionsmöglichkeiten beim Zervixkarzinom
19.05.2009
Host: Brakhage AA, Zipfel PF, Stelzner A

Reuben J. Peters
Max-Planck-Institut für chemische Ökologie, Jena
To Gibberellins and Beyond! Investigating Labdane-Related Diterpenoid Biosynthesis in Plants and Pathogens
09.06.2009
Host: Hertweck C

Jon Clardy
Harvard Medical School, Harvard University, Boston, USA
Lessons from Bugs – The Chemistry of Insect-Bacteria Mutualisms
24.06.2009
Host: Hertweck C

Mark Brönstrup
Sanofi-Aventis Deutschland GmbH
Natural Product Research at Sanofi-Aventis
07.07.2009
Host: Brakhage AA

David Martin
Tepha Medical Devices Inc., Lexington, USA
TephaFLEX® Absorbable Sutures: From DNA to Medical Device
08.09.2009
Host: Roth M

Peter Neubauer
Technische Universität Berlin
EnBase - controlled growth in microtiter plates for high throughput screening and bioprocess scale up
22.09.2009
Host: Horn U

Dietmar G. Forstmeyer
Boeters & Lieck Patentanwälte, München
Erfindungen und Schutzrechte am HKI
13.10.2009
Host: Ramm M

Andreas Stallmach
Universitätsklinikum Jena
Bakterielle Störungen im Gastrointestinaltrakt
20.10.2009
Host: Brakhage AA, Zipfel PF, Stelzner A

Hans-R. Figulla, Maria Wartenberg
Universitätsklinikum Jena
Stammzellen in der Forschung - und in der Klinik (?)
01.12.2009
Host: Brakhage AA, Zipfel PF, Stelzner A

Scientific Awards 2008
Preise und Auszeichnungen 2008

Almeida, Ricardo S.C.
medac-Forschungspreis
medac GmbH, Wedel

Bergmann, Sebastian
Posterpreis des Kongresses "New Directions in Molecular Genetics and Genomics", Freiburg

Brunke, Sascha
medac-Forschungspreis
medac GmbH, Wedel

Habicht, Gernot
medac-Forschungspreis
medac GmbH, Wedel

Haupt, Katrin
medac-Forschungspreis
medac GmbH, Wedel

Haupt, Katrin
medac-Forschungspreis
medac GmbH, Wedel

Hebecker, Mario
Trainee Award of the XXII International Complement Workshop

Heyken, Antje
medac-Forschungspreis
medac GmbH, Wedel

Hortschansky, Peter
medac-Forschungspreis
medac GmbH, Wedel

Hube, Bernhard
Heinz-Maurer-Preis für Dermatologische Forschung (Bereich Klinische Forschung)
Sebapharma GmbH, Boppard

Hube, Bernhard
Fellowship of the American Academy of Microbiology (AAM)

Jacobsen, Ilse D.
Posterpreis beim 1. Leipziger Human- und Tiermedizin-Symposium „Infektion und Immunität: Forschung, Entwicklung und Anwendung", Leipzig

Knüpfer, Uwe
medac-Forschungspreis
medac GmbH, Wedel

Kurzai, Oliver
Förderpreis
Deutsche Gesellschaft für Hygiene und Mikrobiologie DGHM

Lauer, Nadine
Posterpreis auf dem 4th Pro Retina Research-Kolloquium, Potsdam

Partida-Martinez, Laila
Posterpreis der Jahrestagung der VAAM, Frankfurt/M.
VAAM

Partida-Martinez, Laila
DECHEMA-Promotionspreis
DECHEMA

Reuter, Michael
Posterpreis auf dem 4th International Infection Biology Meeting, Malmö, Sweden

Reuter, Michael
medac-Forschungspreis
medac GmbH, Wedel

Richter, Martin
medac-Forschungspreis
medac GmbH, Wedel

Scharf, Daniel H.
medac-Forschungspreis
medac GmbH, Wedel

Scharf, Daniel H.
Posterpreis der Jahrestagung der VAAM, Frankfurt/M.
VAAM

Shelest, Ekaterina
medac-Forschungspreis
medac GmbH, Wedel

Spröte, Petra
medac-Forschungspreis
medac GmbH, Wedel

Traitcheva, Nelly
medac-Forschungspreis
medac GmbH, Wedel

Vödisch, Martin
Promega Award für Wissenschaftsjournalismus
PROMEGA

Vödisch, Martin
Hans Rieth-Posterpreis der 42. Jahrestagung der Deutschsprachigen Mykologischen Gesellschaft in Jena
DMykG

Wieligmann, Karin
medac-Forschungspreis 2008
medac GmbH, Wedel

Winkler, Robert
Promotionspreis anlässlich der der Jahrestagung der VAAM, Frankfurt/M.
VAAM

Wolke, Sandra
medac-Forschungspreis
medac GmbH, Wedel

Zipfel, Peter F.
Heinz-Spitzbart-Preis der European Society for Infectious Diseases in Obstetrics and Gynaecology (ESIDOG)

Zipfel, Peter F.
Lecture Award for the Immunology Letters (efis) at the Annual Meeting of the Hungarian Society for Immunology, Budapest, Hungary

Scientific Awards 2009
Preise und Auszeichnungen 2009

Almeida, Ricardo
Wissenschaftspreis der Stiftung der Deutschsprachigen Mykologischen Gesellschaft
DMykG

Brock, Matthias
Dr. Manfred Plempel Stipendium der Stiftung der Deutschsprachigen Mykologischen Gesellschaft
DMykG

Brock, Matthias
Wissenschaftspreis für Lebenswissenschaften und Physik des Beutenberg-Campus Jena e.V., Kategorie Nachwuchswissenschaftler
Beutenberg-Campus Jena e.V.

Busch, Benjamin
medac-Forschungspreis
medac GmbH, Wedel

Dahse, Hans-Martin
medac-Forschungspreis
medac GmbH, Wedel

Enghardt, Tina
medac-Forschungspreis
medac GmbH, Wedel

Gropp, Katharina
medac-Forschungspreis
medac GmbH, Wedel

Grumbt, Maria
Young Outstanding Investigator Award des 3. FEBS Advanced Lecture Course "Human Fungal Pathogens"
FEBS

Hälbich, Steffi
medac-Forschungspreis
medac GmbH, Wedel

Hallström, Teresia
Best oral presentation on the 12th European Meeting on Complement in Human Disease, Visegrad, Hungary
12th European Meeting on Complement in Human Disease

Hartmann, Andrea
medac-Forschungspreis
medac GmbH, Wedel

Heinen, Stefan
medac-Forschungspreis
medac GmbH, Wedel

Jacobsen, Ilse D.
Posterpreis Grundlagenforschung und Diagnostik der Stiftung der Deutschsprachigen Mykologischen Gesellschaft
DMykG

Kusebauch, Björn
medac-Forschungspreis
medac GmbH, Wedel

Lauer, Nadine
medac-Forschungspreis
medac GmbH, Wedel

Lauer, Nadine
Posterpreis auf dem 5th Pro Retina Research-Kolloquium
5th Pro Retina Research-Kolloquium

Lüttich, Anja
Posterpreis auf dem 1. Symposium der International Leibniz Research School/Jenaer School for Microbial Communications, Jena
JSMC/ILRS

Martin, Karin
medac-Forschungspreis
medac GmbH, Wedel

Martin, Ronny
Posterpreis der Firma Bayer Vital anlässlich des internationalen Kongresses „Sepsis und Multiorganversagen"
Firma Bayer Vital

Mihlan, Michael
medac-Forschungspreis
medac GmbH, Wedel

Nützmann, Hans-Wilhelm
medac-Forschungspreis
medac GmbH, Wedel

Roth, Martin
medac-Forschungspreis
medac GmbH, Wedel

Scharf, Daniel
Examenspreis der Biologisch-Pharmazeutischen Fakultät
FSU Jena

Scherlach, Kirstin
medac-Forschungspreis
medac GmbH, Wedel

Schmidt-Heck, Wolfgang
medac-Forschungspreis
medac GmbH, Wedel

Schroeckh, Volker
medac-Forschungspreis
medac GmbH, Wedel

Schümann, Julia
medac-Forschungspreis
medac GmbH, Wedel

Shelest, Ekatarina
medac-Forschungspreis
medac GmbH, Wedel

Skibbe, Melanie
Posterpreis Grundlagenforschung und Diagnostik der Deutschsprachigen Mykologischen Gesellschaft
DMykG

Staib, Peter
Heinz P.R. Seeliger Preis der Seeliger-Stiftung
Seeliger-Stiftung

Stippa, Selina
medac-Forschungspreis
medac GmbH, Wedel

Thön, Marcel
Best poster presentation of the meeting of the Xth International Fungal Biology Conference Ensenada, Mexico
Xth International Fungal Biology Conference

Wiehl, Ulrike
medac-Forschungspreis
medac GmbH, Wedel

Wilson, Duncan
Best Talk Prize Formedium at the Annual Meeting of the British Mycological Society (BMC), Dundee, Scotland
Firma Formedium

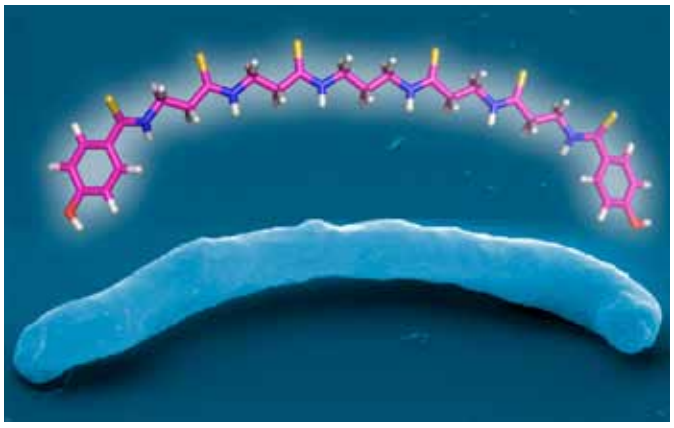
Wilson, Duncan
Posterpreis "Young Investigator Award" des Journals Cell Host & Microbe und "Young Outstanding Investigator Award" des Journals Eukaryotic Cell beim 3. FEBS Advanced Lecture Course "Human Fungal Pathogens"
Zeitschriften *Cell Host & Microbe* und *Eukaryotic Cell*

Zipfel, Peter F.
Hauptpreis der Deutschen Gesellschaft für Hygiene und Mikrobiologie
DGHM

Inventions and Patents 2008/2009
Erfindungen und Schutzrechte 2008/2009

In addition to publications in peer reviewed journals, intellectual property rights are main performance parameters reflecting the quality of research at the HKI. In 2008/2009 a remarkable number of inventions were filed both by departments focusing on natural product research and by technology-oriented groups, thus broadening the patent-portfolio of the institute. HKI patents resulted in a number of fruitful co-operations with industry and affected the institute's budget advantageously.
A group of HKI scientists led by Prof. Christian Hertweck discovered the first secondary metabolite from a strictly anaerobic bacterium, *Clostridium cellulolyticum*. The substance – named closthioamide – possesses a highly unusual symmetric structure containing numerous sulphur atoms. Biological profiling by the Internal Product Line revealed that the new molecule shows strong antibacterial activity against multiresistant pathogens. Presently, this promising lead structure is being developed into a drug candidate by chemical and molecular biological methods.
The application for new patents is stringently evaluated within the HKI and focuses on novel biologically active natural products and their (bio-)synthetic derivatives. Since 2006 the HKI co-operates with Ascenion GmbH, Munich, to further optimize technology transfer and commercialization activities. This project is funded by the Federal Ministry of Education and Research.

Schutzrechte sind neben Originalpublikationen in referierten Fachjournalen ein wesentlicher Leistungsparameter für die Forschungsarbeit am HKI. Sowohl die naturstoffchemisch arbeitenden Abteilungen als auch Technologie-orientierte Gruppen trugen im Zeitraum 2008/2009 mit einer Reihe 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.
Eine Gruppe von Wissenschaftlern aus dem HKI entdeckte unter Leitung von Prof. Christian Hertweck den ersten sekundären Naturstoff bei einem strikt anaeroben Bakterium. Die neue Substanz – sie erhielt die Bezeichnung Closthioamid – ist ein strukturell sehr ungewöhnliches, symmetrisches Molekül, das zahlreiche Schwefelatome besitzt. Die biologische Profilierung in der Durchgehenden Bearbeitungslinie des HKI zeigte zudem, dass Closthioamid gegen multiresistente Krankheitserreger aktiv ist. Derzeit wird die Leitstruktur mit chemischen und molekularbiologischen Methoden zu einem Arzneistoffkandidaten weiterentwickelt.
Die Anmeldung neuer Schutzrechte unterliegt einer strengen hausinternen Evaluation und konzentriert sich auf neue, biologisch aktive Naturstoffe und deren (bio-)synthetische Derivate. In einem BMBF-geförderten Projekt zur effektiven Verwertung der Schutzrechte arbeitet das HKI seit 2006 erfolgreich mit der Ascenion GmbH, München zusammen.



In the presence of soil extracts the obligate anaerobe *Clostridium cellulolyticum* produces a symmetric molecule with a central diaminopropyl moiety connected by polythioamide linkers to terminal p-hydroxybenzoyl groups. This compound, named closthioamide, was found to have antibiotic activity against methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant enterococci.

Hertweck C, **Werneburg M**, **Roth M**, **Dahse HM** (2008)
Novel Aureothin Derivatives
EP 08 007 043.6

Brock M, Ibrahim-Granet O (2008)
Bioluminescent Strain *Aspergillus fumigatus*
PCT/EP2009/062340

Zipfel PF, **Skerka C**, Wallich
Neue Regulatoren des angeborenen Immunsystems
DE 10 2008 049 136.5, PCT/EP2009/006963

Linde K, Große-Herrenthey A, Krüger M, Büsing K, **Jacobsen I**
Impfstämme gegen bakterielle und fungale Infektionen.
DE 10 2008 062 9413

Deigner HP, Russwurm S, **Saluz HP** (2009)
Control genes for the normalization of gene expession analysis data
EP20080716110

Abdou R, **Dahse HM**, **Hertweck C**, **Scherlach K**, **Sattler I** (2009)
Botryosphaerones, novel depsidones and their use as medicaments
EP2230235

Behnken S, **Dahse HM**, **Hertweck C**, **Ishida K**, **Kloß F**, **Lincke T**, **Roth M** (2009)
Closthioamides
EP09013674

Meetings, Workshops, Symposia 2008/2009
Wissenschaftliche Veranstaltungen 2008/2009

Irseer Naturstofftage
Hertweck C et al.
Irsee, Germany
2008

SIM Meeting (Natural Products Session)
Hertweck C et al.
San Diego, USA
2008

42. Wissenschaftliche Tagung der Deutschsprachigen Mykologischen Gesellschaft e.V. (DGMyk)
Brakhage AA, Hipler U-C, **Hube B**, **Zipfel PF**, **Brock M**
Jena, Germany
2008

ILRS Symposium
Schmidt D, **Zipfel PF**
Dornburg/Jena, Germany
2008

ILRS Group Seminar
Brakhage AA, **Schmidt D**
Jena, Germany
2008

DFG-SPP 1160 Statusworkshop
Brakhage AA, **Kniemeyer O**
Jena
2008

Photonics meets Life Science
Popp J, **Brakhage AA**, Boland W
Jena, Germany
2008

International Workshop Droplet-based microfluidics for high-throughput applications in life sciences
Brakhage AA, **Roth M**
Jena, Germany
2008

Invasive Mycoses in Hematological Malignancies II
Kurzai O et al.
Würzburg, Germany
2008

International Workshop Transcriptome and Proteome Data Analysis and Warehousing towards Systems Biology
Guthke R et al.
Stuttgart, Germany
2008

International Workshop Gene Regulatory Network Inference
Guthke R et al.
Jena, Germany
2008

Irseer Naturstofftage
Hertweck C et al.
Irsee, Germany
2009

ILRS Group Seminar
Schmidt D, **Zipfel PF**
Jena, Germany
2009

ILRS Group Seminar
Schmidt D, **Zipfel PF**
Jena, Germany
2009

4th International Workshop on Thrombotic Microangiopathies
John U, Kentouche K, Wolf G, **Skerka C**, **Zipfel PF**
Weimar, Germany
2009

Joint ILRS/JSMC Symposium
Schmidt D, **Zipfel PF**, **Brakhage AA**, Boland W, Kothe E, Thoms C
Jena, Germany
2009

4th International Congress "Sepsis and Multiorgan Dysfunction"
Brakhage AA, Reinhart K
Weimar, Germany
2009

DFG-SPP1160 Meeting
Brakhage AA, **Kniemeyer O**
Jena, Germany
2009

Invasive Mycoses in Hematological Malignancies III
Kurzai O et al.
Würzburg, Germany
2009

Jahrestagung der Deutschen Pharmazeutischen Gesellschaft DPhG 2009
Hoffmeister D et al.
Jena, Germany
2009

International Workshop on Integrative Network Inference in Systems Biology
Guthke R et al.
Jena, Germany
2009

Living with pathogens – never lose control (International Symposium of the DFG Collaborative Research Centre 479)
Kurzai O et al.
Würzburg, Germany
2009

Participation in Research Networks 2008/2009
Beteiligung an Netzwerken und Verbundprojekten 2008/2009

Exzellenz-Graduiertenschulen der Deutschen Forschungsgemeinschaft

Jena School for Microbial Communication JSMC
Teilprojekt 3: "Criss-Cross" talk between filamentous fungi and streptomycetes
Axel Brakhage/Volker Schroeckh
Laufzeit: 11/2007 – 03/2011

Jena School for Microbial Communication JSMC
Teilprojekt 4: Interaction of *Candida albicans* and host cells: Fungal nutrient acquisition and host defense mechanisms
Matthias Brock
Laufzeit: 11/2007 – 03/2011

Jena School for Microbial Communication JSMC
Teilprojekt 6: Molecular basis of bacterial-fungal symbioses
Christian Hertweck
Laufzeit: 11/2007 – 09/2012

Jena School for Microbial Communication JSMC
Teilprojekt 9: Crosstalk between innate pathogen sensing molecules
Mihály Józsi
Laufzeit: 11/2007 – 04/2011

Jena School for Microbial Communication JSMC
Teilprojekt 10: Development of a procedure to avoid misincooperation of rare amino acids into therapeutic proteins during high cell density fermentation
Uwe Horn
Laufzeit: 11/2007 – 10/2011

Jena School for Microbial Communication JSMC
Teilprojekt 19: Immunevasion of pathogenic microbes
Peter Zipfel
Laufzeit: 07/2008 – 06/2011

Jena School for Microbial Communication JSMC
Teilprojekt 21: Host pathogen interactions of human pathogenic yeast
Bernhard Hube
Laufzeit: 10/2008 – 01/2012

Jena School for Microbial Communication JSMC
Teilprojekt 26: Systems biology of human pathogenic fungi: Modeling transcriptional networks of virulence
Reinhard Guthke
Laufzeit: 10/2008-10/2011

Jena School for Microbial Communication JSMC
Teilprojekt 31: Comparative genomics of host/chlamydiales interactions
Hans Peter Saluz
Laufzeit: 10/2008 – 01/2012

Jena School for Microbial Communication JSMC
Teilprojekt 34: Analysis of environmental signalling and morphogenetic control in the human pathogenic yeast *Candida albicans*
Peter Staib
Laufzeit: 10/2008 – 11/2011

Jena School for Microbial Communication JSMC
Teilprojekt 39: Analysis of the interaction of the human pathogenic fungus *Aspergilllus fumigatus* with immune effector cells by functional genomics
Axel Brakhage
Laufzeit: 09/2009 – 12/2012

Sonderforschungsbereiche der Deutschen Forschungsgemeinschaft

Sonderforschungsbereich 604: Multifunktionelle Signalproteine
Teilprojekt B02: Regulation of DNA polymerase alpha, Cdc45 and TopBP1 at the Initiation step of DNA replication
Frank Hänel
Laufzeit: 07/2005 – 12/2009

Schwerpunktprogramme der Deutschen Forschungsgemeinschaft

Schwerpunktprogramm 1152: Evolution metabolischer Diversität
Nicht-colineare Thiotemplat-Systeme als Modell für die Evolution von Polyketidsynthasen
Christian Hertweck
Laufzeit: 01/2008 – 12/2010

Schwerpunktprogramm 1160: Kolonisation und Infektion durch humanpathogene Pilze
Koordination
Axel Brakhage
Laufzeit: 08/2008 – 12/2010

Schwerpunktprogramm 1160: Kolonisation und Infektion durch humanpathogene Pilze
Teilprojekt: Immune evasion mechanisms of the human pathogenic yeast *Candida albicans*
Peter Zipfel
Laufzeit: 09/2008 – 12/2010

Schwerpunktprogramm 1160: Kolonisation und Infektion durch humanpathogene Pilze
Teilprojekt: Carbon-and nitrogen utilisation of pathogenic *Candida* and *Aspergillus* species during pathogenesis
Matthias Brock
Laufzeit: 08/2008 – 12/2010

Schwerpunktprogramm 1160: Kolonisation und Infektion durch humanpathogene Pilze
Teilprojekt: Identification and characterisation of virulence associated genes during oral infections with *Candida albicans*
Bernhard Hube
Laufzeit: 08/2008 – 12/2011

Schwerpunktprogramm 1160: Kolonisation und Infektion durch humanpathogene Pilze
Teilprojekt: Identification of virulence determinants of the human-pathogenic fungus *Aspergillus fumigatus* by proteome analysis
Axel Brakhage
Laufzeit: 08/2008 – 12/2010

Schwerpunktprogramm 1160: Kolonisation und Infektion durch humanpathogene Pilze
Teilprojekt: Holistic approach to genomics of human-pathogenic fungi: Data warehouse for integration of data on transcriptome, proteome and metabolome of *Candida albicans* and *Aspergillus fumigatus*
Axel Brakhage / Reinhard Guthke
Laufzeit: 08/2008 – 07/2010

Schwerpunktprogramm 1160: Kolonisation und Infektion durch humanpathogene Pilze
Teilprojekt: Molecular analysis of the poly-telomeric CTA2 gene family of *Candida albicans*: Elucidation of genetic or epigenetic regulation of their differential expression, localisation and functional analyses *in vitro* and during the early phase of colonisation on porcine intestinal epithelium
Jürgen Wendland
Laufzeit: 07/2006 – 06/2008

EU-Projekte

Programme Information Society Technologies FP6/2002/IST/1
Teilprojekt: Nature-inspired Smart Information Systems
Reinhard Guthke
Laufzeit: 01/2005 – 01/2008

Integrated Project: New medicines for tuberculosis (NM4TB) (FP6)
Ute Möllmann
Laufzeit: 01/2006 – 12/2008

Integrating and strengthening the European Research Area Specific Targeted Project MANASP (FP6)
Development of novel management strategies for invasive aspergillosis
Axel Brakhage / Olaf Kniemeyer
Laufzeit: 12/2006 – 05/2010

Marie Curie Research Training Networks SIGNALPATH (FP6)
MAP kinase cascades controlling virulence in fungi: from signals to pathogenicity response
Axel Brakhage / Thorsten Heinekamp
Laufzeit: 11/2005 – 10/2009

Marie Curie Research Training Networks CaInfectome (FP7)
Unravelling the infectome of *Candida albicans*
Bernhard Hube
Laufzeit: 05/2008 – 05/2010

Marie Curie Research Training Networks FINSysB (FP7)
Pathogenomics and systems biology of fungal infections - an integrative approach
Bernhard Hube
Laufzeit: 11/2008 – 10/2012

Verbundvorhaben des Bundesministeriums für Bildung und Forschung

ZIK Septomics
Forschungsgruppe Fungal Septomics: Molekulare Mechanismen in der Pathogenese der Sepsis durch Pilze
Axel Brakhage / Oliver Kurzai
Laufzeit: 11/2009 – 10/2014

GenomikPlus
Teilprojekt: Neue antibakterielle und antitumorale Polyketide durch Biokombinatorik
Christian Hertweck
Laufzeit: 08/2006 – 12/2009

BioChancePLUS-2: Verbundprojekt: Individualisierte Medizin: Tool zur Therapieentscheidung – Apherese/Immunadsorption-Chip
Teilprojekt 4: Bioinformatische Analysen der RA-Patienten: Identifizierung von Genen und Genprodukten für das Ansprechverhalten zur Apherese-Immunadsorptionstherapie
Reinhard Guthke
Laufzeit: 09/2006 – 01/2010

HepatoSys-Systembiologie regenerierender Hepatozyten
Teilprojekt B3: Dynamische Modellierung des Wnt/beta-Catenin Signalweges während der Leberregeneration
Reinhard Guthke
Laufzeit: 01/2007 – 12/2009

SERIZELL
Teilprojekt: Systemevaluierung mit ausgewählten Bioassays
Martin Roth
Laufzeit: 01/2005 – 06/2008

Technologietransfer
Verstetigung der Verwertungskonzepte in den lebenswissenschaftlichen Instituten der WGL – Leibniz-Institut für Naturstoff-Forschung und Infektionsbiologie e.V. – Hans-Knöll-Institut - (HKI)
Michael Ramm
Laufzeit: 01/2009 – 03/2012

ERA-Net PathoGenoMics-FunPath
Genomweite Ansätze zur Aufklärung der molekularen Pathogenitätsmechanismen des Humanpathogens *Candida glabrata*
Bernhard Hube
Laufzeit: 02/2007 – 03/2010

ERA-NET PathoGenoMics-Apergillosis
Die Zellwand als Target zur Verbesserung der antifungalen Therapie der Aspergillose
Olaf Kniemeyer
Laufzeit: 02/2009 – 05/2012

SPICE
Schnelle Diagnose und Überwachung von Shrimpkrankheiten und Shrimppathogenen in natürlicher Umgebung und in der Aquakultur
Hans Peter Saluz
Laufzeit: 08/2007 – 12/2010

Zoonotische Chlamydien
Molekulare Pathogenese: Charakterisierung der Virulenz zoonotischer Chlamydien und der Wirtszellreaktion nach Infektion – vergleichende Transkriptom-, Proteom- und Interaktom-Untersuchungen
Hans Peter Saluz / Frank Hänel
Laufzeit: 09/2007 – 12/2010

INTERCOMMUNIC(A)TE
Integrierte Suche nach neuen Wirkstoffen aus Mikroorganismen aus einzigartigen chinesischen Lagerstätten
Christian Hertweck
Laufzeit: 04/2008 – 12/2011

PET-CT
PET/CT zur Darstellung von Infektion und Entzündung: Reduction, Refinement und Replacement von Tierversuchen
Hans Peter Saluz / Ilse Jacobsen
Laufzeit: 05/2008 – 09/2011

FORSYS
Teilprojekt 5: Dynamik und Regulation der metabolischen Balance in *E. coli*
Reinhard Guthke
Laufzeit: 07/2008 – 06/2011

JenAge
Jenaer Centrum für die Systembiologie des Alterns - JenAge: Systembiologie von mildem Stress beim gesunden Altern ein Multi-Spezies-Ansatz
Reinhard Guthke
Laufzeit: 10/2009 – 09/2012

DiNaMid
Genom-basierte Findung neuer antimikrobieller Naturstoffe in mikrofluidischen Chips; TP: Identifizierung neuer Stoffwechselwege und Naturstoff-Isolierung
Markus Nett / Martin Roth
Laufzeit: 10/2009 – 09/2012

Weitere Verbundvorhaben

German Israeli Foundation for Scientific Research & Development
Focusing on Hormone-Independent Breast Cancer
Christian Hertweck
Laufzeit: 01/2006 – 12/2008

National Institutes of Health (NIH)
Novel Derivatization / Functionalization of Natural Products
Ute Möllmann
Laufzeit: 09/2005 – 07/2008

Bundesumweltamt
Untersuchungen zum Vorkommen und zur gesundheitlichen Relevanz von Bakterien in Innenräumen
Ingrid Groth
Laufzeit: 12/2005 – 05/2008

Calls for Appointments 2008/2009
Rufe 2008/2009

Christian Hertweck
Professor für Naturstoffchemie an der Friedrich-Schiller-Universität Jena
Ruf an die Technische Universität München (2008)
– abgewendet –

Laila Partida-Martinez (2008)
Assistant Professor
University of Monterrey, Mexico

Robert Winkler (2008)
Assistant Professor
University of Monterrey, Mexico

Dirk Hoffmeister (2009)
Professor für Pharmazeutische Biologie
Biologisch-Pharmazeutische Fakultät der Friedrich-Schiller-Universität Jena

Oliver Kurzai (2009)
Professur für Fungal Septomics
Friedrich-Schiller-Universität Jena

Postdoctoral Lecture Qualifications 2008/2009
Habilitationen 2008/2009

Kurzai, Oliver (2008)
Vom Kommensalen zum Krankheitsserreger – mikrobielle und immunologische Voraussetzungen für Virulenzvariation
Julius-Maximilians-Universität Würzburg

Graduations 2008/2009
Promotionen 2008/2009

2008

Al-Abdallah, Qusai
Regulation ot the CCAAT-binding complex of *Aspergillus nidulans* under oxidative stress and iron-depleting conditions
Friedrich-Schiller-Universität Jena

Almeida, Ricardo S.C.
Die Rolle von Eisen für die Virulenz von *Candida albicans* bei oralen Infektionen
Friedrich-Schiller-Universität Jena

Haupt, Katrin
Rekrutierung von Komplementregulatoren der Faktor H Proteinfamilie als Mechanismen der Immunevasion humanpathogener Erreger
Friedrich-Schiller-Universität Jena

Ibanez, Alfredo
Development of a polymeric planar microwell device (pMALDI chip) for enhancing protein analysis in combination with MALDI-TOF/MS instrumentation
Friedrich-Schiller-Universität Jena

Leßing, Franziska
Untersuchungen zu den molekularen Mechanismen der Stressantwort von *Aspergillus fumigatus* bei Interaktion mit Immuneffektorzellen
Friedrich-Schiller-Universität Jena

Mitra, Sirsha
Herbivore induced changes in the plant's proteome and the role of atypical herbivore-responsive proteins in *Nicotiana attenuata*
Friedrich-Schiller-Universität Jena

Schmidt, Uta
Das multifunktionelle Signalprotein Topoisomerase IIß-Bindeprotein 1 (TopBP1) und seine Funktion in der DNA-Schadenserkennung und Replikation
Friedrich-Schiller-Universität Jena

Siegemund, Martin
Analyse der Protein-Transphosphorylierungsaktivität der Phosphatidylinositol 3-Kinase Vps34p aus *Candida albicans*
Friedrich-Schiller-Universität Jena

Spröte, Petra
Regulation and evolution of the penicillin biosynthesis gene cluster of *Aspergillus nidulans*
Friedrich-Schiller-Universität Jena

Villwock, Andrea
Bedeutung des Klasse A Scavenger Rezeptors für die Zytokinsekretion von humanen dendritischen Zellen nach Kontakt mit dem humanen Pathogen *Neisseria meningitidis*
Julius-Maximilians-Universität Würzburg

Werneburg, Martina
Chemo-Biosynthese von Aureothin und verwandten Pyronverbindungen
Friedrich-Schiller-Universität Jena

Wozniok, Iwona
Interaktion von humanen Granulozyten mit den Pilzen *Candida albicans* und *Aspergillus fumigatus*
Universität Stuttgart

Zöllner, Tina
Chemische Derivatisierung des Makrolid-Antibiotikums Leucomycon
Friedrich-Schiller-Universität Jena

2009

Gehrke, Alexander
Charakterisierung heptahelikaler Rezeptoren in *Aspergillus fumigatus*
Friedrich-Schiller-Universität Jena

Misiek, Mathias
Untersuchung der Armillylorsellinat-Biosynthese aus dem Phytopathogen *Armillaria mellea* zur einfachen Evaluation seiner Virulenz
Albert-Ludwigs-Universität Freiburg

Schmaler-Ripcke, Jeannette
Melanin in the human pathogenic fungus *Aspergillus fumigatus*: Discovery of a novel melanin in the fungus and the use of conidial melanin as a target for camelid heavy-chain antibodies
Friedrich-Schiller-Universität Jena

Schneider, Patrick
Untersuchung der Biosynthese von Terrechinon A, Atromentin und Ralfuranon
Albert-Ludwigs-Universität Freiburg

Schümann, Julia
Identifikation and Manipulation of Fungal PKS-NRPS Hybrid Pathways
Friedrich-Schiller-Universität Jena

Shroff, Rohit
Enhancing MALDI for Metabolomic Analysis: Novel Matrices and Molecular Imaging
Friedrich-Schiller-Universität Jena

Trinh Thi Tam, Bao
Response of human leukemia cell upon treatment with bioactive extracts from tropical medical mushrooms
Friedrich-Schiller-Universität Jena

Bachelor / Master / Diploma Theses 2008/2009

Bachelor- / Master- / Diplomarbeiten 2008/2009

2008

Bechstein, Stefanie
Funktionsanalyse von pilzlichen PKS/NRPS-Hybridsynthasen
Friedrich-Schiller-Universität Jena

Biedermann, Gesine
Untersuchung der Expression des Gens *yvcC* in *Bacillus subtilis* Wildtyp und der Cervimycin C-resistenten Mutante 8R
Fachhochschule Jena

Böhme, Julia
Rolle von Proteinen der Faktor H Familie bei der alterabhängigen Makuladegeneration
Friedrich-Schiller-Universität Jena

Brendel, Nicole
Untersuchung der Rhizoxin-Biosynthese in *Pseudomonas fluorescens* Pf-5 und *Burkholderia rhizoxinica* B1
Friedrich-Schiller-Universität Jena

Eberhardt, Hannes
Charakterisierung des Complement related proteins 2 (CFHR2)
Friedrich-Schiller-Universität Jena

Enghardt, Tina
Komplement Immunevasion bei Krebszellen
Friedrich-Schiller-Universität Jena

Fries, Alexander
Mutagenese- und Aktivitätsstudien an AurF, einer Mn-abhängigen *N*-Oxygenase
Friedrich-Schiller-Universität Jena

Grützmann, Konrad
Rekonstruktion von Genregulationsnetzwerken bei *Candida albicans*
Friedrich-Schiller-Universität Jena

Kaleta, Christoph
Inference of Gene Regulatory Networks in *Escherichia coli*.
Friedrich-Schiller-Universität Jena

Köhler, Katja
Molekulare Mechanismen der Immunevasion von *Pseudomonas aeruginosa*
Friedrich-Schiller-Universität Jena

Litsche, Katrin
Untersuchung der Wirkung des bioaktiven *Phellinus*-Pilzextrakts auf Protein- und microRNA-Expression in menschlichen Tumorzellen
Friedrich-Schiller-Universität Jena

Mech, Franziska
Bestimmung optimaler Probesets mittels boolescher Terme
Friedrich-Schiller-Universität Jena

Münzberg, Christin
Immunevasionmechanismen der humanpathogenen Hefe *Candida albicans*
Friedrich-Schiller-Universität Jena

Scharf, Daniel
Identifizierung und Charakterisierung Yap-ähnlicher Transkriptionsfaktoren aus *Aspergillus nidulans*
Friedrich-Schiller-Universität Jena

Steinbrücker, Carolin
Aufreinigung und Charakterisierung des eisenabhängigen Transkriptionsfaktors HapX in *Aspergillus nidulans*
Friedrich-Schiller-Universität Jena

Steube, Arndt
UV-Laser-X-ChIP Display zum Studium von Nukleoprotein Komplexen *in vivo*
Friedrich-Schiller-Universität Jena

Vogel, Annette
Vergleichende Proteomik von *Chlamydophila spp.* infizierten BGM-Zellen
Friedrich-Schiller-Universität Jena

Wartenberg, Dirk
Identifizierung und Charakterisierung der sekretierten Proteine des humanpathogenen Pilzes *Aspergillus fumigatus*
Friedrich-Schiller-Universität Jena

Wiehl, Ulrike
Funktionelle Charakterisierung der Faktor H-verwandten Proteine 1 und 3 (FHR-1 und FHR-3)
Friedrich-Schiller-Universität Jena

Wolf, Katharina
Funktionelle Analyse von vier potentiellen chlamydialen *(Chlamydophila pneumoniae)* Apoptose-Inhibitoren
Friedrich-Schiller-Universität Jena

Wolke, Sandra
Proteom- und molekulargenetische Analyse von *Aspergillus fumigatus* und *A. nidulans*
Friedrich-Schiller-Universität Jena

2009

Böhm, Sascha
Vergleichende Charakterisierung fusionierter Komplementinhibitoren von *Staphylococcus aureus*
Friedrich-Schiller-Universität Jena

Boland, Sebastian
Analyse von NRPS-Gen-Clustern in endofungalen Bakterien
Friedrich-Schiller-Universität Jena

Böttger, Daniela
Molecular Jigsaw with Two Related Fungal PKS-NRPS Hybrids; Engineering Cytochalasan and Lovastatin Biosynthesis
Friedrich-Schiller-Universität Jena

Bretschneider, Tom
Funktionelle Analyse einer Ketosynthase mit ungewöhnlicher Acyltransfer-Funktion
Fachhochschule Jena

Domin, Nicole
Erzeugung biolumineszierender *Aspergillus terreus*- und *Fusarium solani*-Stämme und Untersuchungen zur Propionyl-CoA Detoxifizierung
Friedrich-Schiller-Universität Jena

Fazius, Eugen
Entwicklung und Implementierung von datenbankbasierten Software-Tools für die Vorhersage von Transkriptionsfaktorbindestellen in pathogenen Pilzen
Fachhochschule Bingen

Greßler, Markus
Sekundärmetabolite des filamentösen Pilzes *Aspergillus terreus*: Expressionsanalyse und Produktnachweis
Friedrich-Schiller-Universität Jena

Groh, Katrin
Molekulare Ansätze zum Studium von Shrimp-Pathogen-Interaktionen
Friedrich-Schiller-Universität Jena

Horn, Fabian
Full Genomic Microarray Probe Design for *Aspergillus nidulans*
Friedrich-Schiller-Universität Jena

Keller, Sophia
Regulation des Tyrosinabbaus und der Pyomelanin-Biosynthese in *Aspergillus fumigatus*
Friedrich-Schiller-Universität Jena

König, Claudia
Aktivierung eines stillen Genclusters des humanpathogenen Pilzes *Aspergillus fumigatus* durch Kultivierung mit *Streptomyces rapamycinicus*
Friedrich-Schiller-Universität Jena

Kopka, Isabell
Die Rolle von Apolipoteinen bei der Phagozytose von geschädigten humanen Endothelzellen
Friedrich-Schiller-Universität Jena

Krichel, Boris
Analyse des Auflösungsvermögens eines PET/µCT-Scanners für die Kleintierbildgebung
Fachhochschule Jena

Lapp, Katrin
Funktionelle Charakterisierung der Hämolysine von *Aspergillus fumigatus* und deren Bedeutung für die Pathogenität
Friedrich-Schiller-Universität Jena

Leonhardt, Ines
Charakterisierung der Interaktion von *Candida albicans* mit Neutrophilen mit Hilfe von Mutanten und GFP-Reporterstämmen
Friedrich-Schiller-Universität Jena

Macheleidt, Juliane
Pyomelaninbildung und Proteinkinase A-regulierte Prozesse in dem humanpathogenen Pilz *Aspergillus fumigatus*
Friedrich-Schiller-Universität Jena

Roth, Mareike
Genome-wide profiles of *in vivo* Stat1-DNA interactions using UV laser X ChIP technologies
Fachhochschule Krems, Österreich

Massier, Julia
Untersuchung neuer Interaktionen zwischen chlamydialen Proteinen und humanen Wirtszellproteinen
Friedrich-Schiller-Universität Jena

Müller, Sebastian
Robuste Erkennung von Strukturen in molekularbiologischen Daten auf der Grundlage des Ensemble-Clusterings
Friedrich-Schiller-Universität Jena

Ottwald, Dirk
Studien zur Synthese von Aureothin-Konjugaten
Friedrich-Schiller-Universität Jena

Maiwald, Peter
Das Malat Enzym aus *Aspergillus fumigatus*: Identifizierung der biochemischen Parameter und phänotypische Charakterisierung von Deletionsmutanten
Fachhochschule Jena

Priebe, Steffen
Gütebewertung von Netzwerkinferenz-Verfahren
Friedrich-Schiller-Universität Jena

Rohm, Barbara
Toxinproduktion lebensmittelrelevanter Burkholderien
Friedrich-Schiller-Universität Jena

Rommel, Kerstin
Molekularbiologische Untersuchung der oxidativen Umlagerung in der Chartreusin-Biosynthese
Friedrich-Schiller-Universität Jena

Schau, Virginie
Molekularbiologische und chemische Untersuchungen an einem tropischen Baumpilz
Friedrich-Schiller-Universität Jena

Stelzner, Kristin
Interaktion von Akut-Phase-Proteinen und Komplementproteinen
Friedrich-Schiller-Universität Jena

Miesch, Stephanie
Molekularbiologische Untersuchungen zur Entwicklung eines Transformationssystems für den humanpathogenen Dermatophyten *Trichophyton rubrum*
Fachhochschule Lausitz

Stock, Magdalena
Inferring Gene Regulatory Networks from Microarray Data of *Candida albicans*
Friedrich-Schiller-Universität Jena

Ueberschaar, Nico
Semisynthese von Chartreusin-Derivaten
Friedrich-Schiller-Universität Jena

Uhde, Melanie
Complement evasion strategies of *Pseudomonas aeruginosa*
Friedrich-Schiller-Universität Jena

van der Smissen, Anja
Transkriptionsprofil des humanpathogenen Pilzes *Aspergillus fumigatus* während der Konfrontation mit humanem Blut
Friedrich-Schiller-Universität Jena

Voigt, Jessica
Herstellung chimärer Antigen-Rezeptoren zur tumorspezifischen Armierung von T-Lymphozyten
Technische Universität Dresden

Vollstädt, Sebastian
Optimierung und Standardisierung eines Fermentations- und Downstream-Prozesses zur Herstellung eines Antibiotikums
Hochschule Anhalt (FH), Köthen

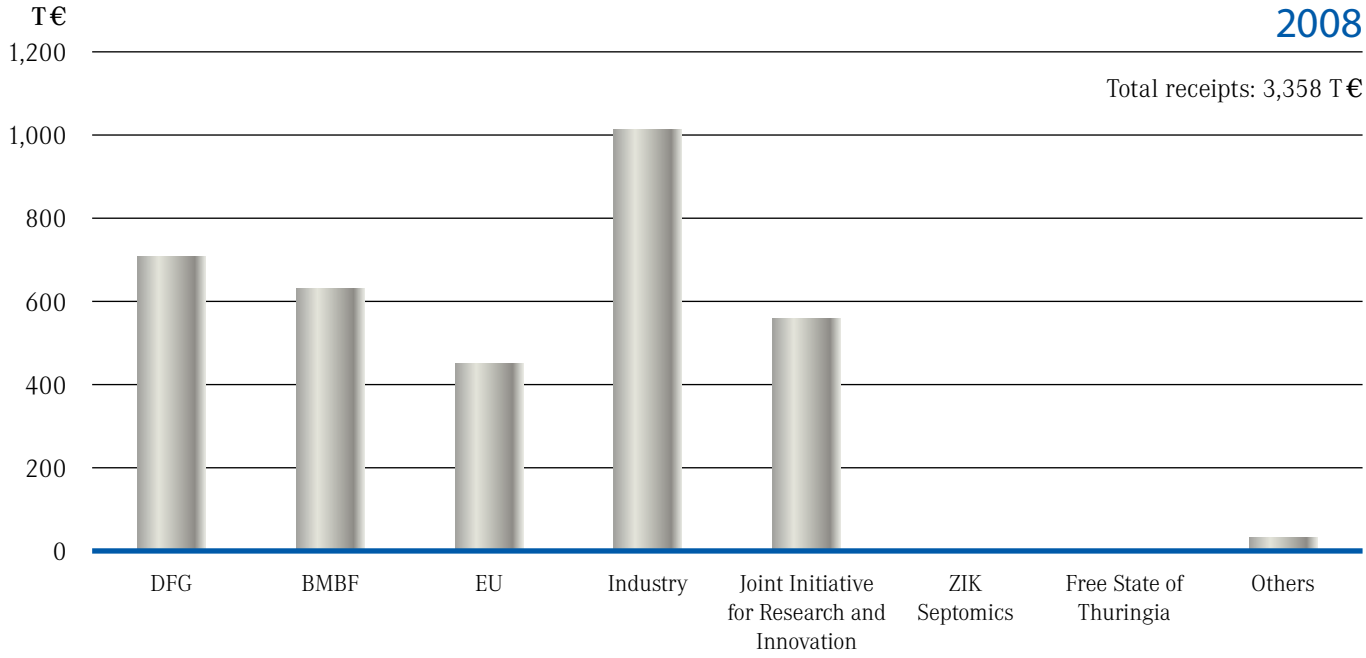
Wackler, Barbara
Genomische Untersuchung zu Biosynthese der Urdamycine C und D in *Streptomyces fradiae*
Albert-Ludwigs-Universität Freiburg

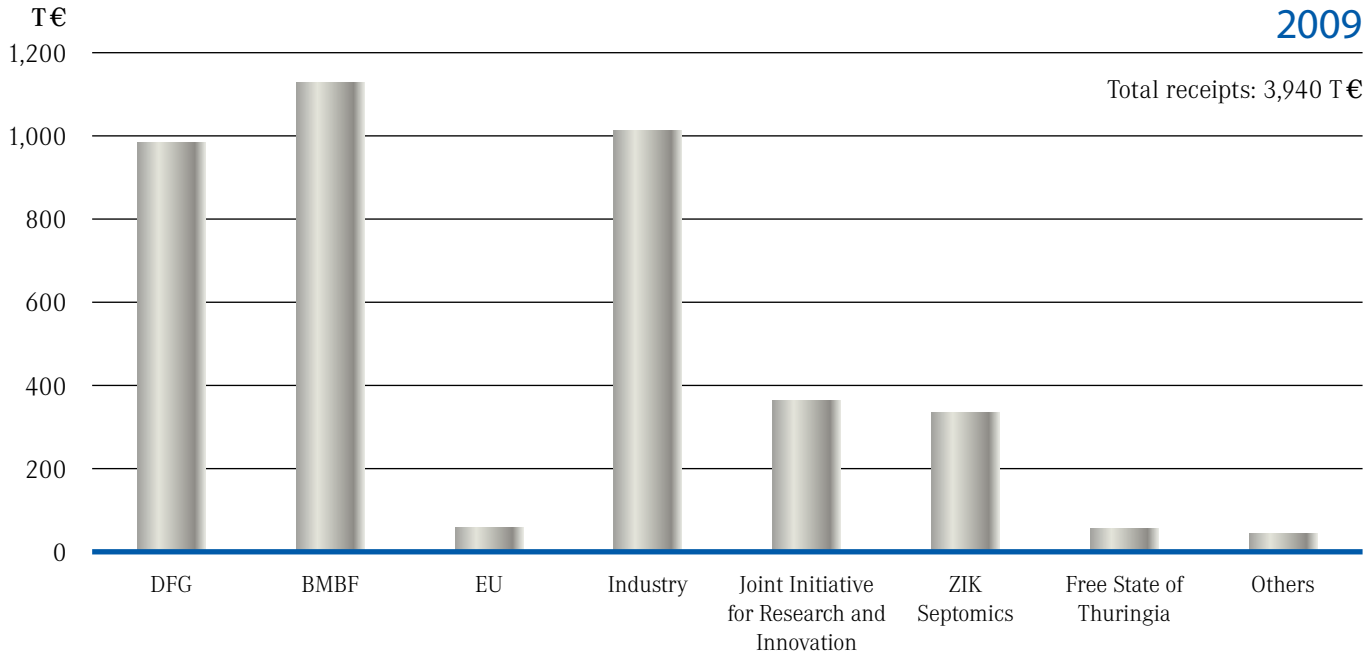
Walther, Elisabeth
Untersuchungen zur genetischen Variabilität von indonesischen *Penaeus monodon* Shrimps
Fachhochschule Jena

Weber, Nadia
Klonierung und Charakterisierung von Apolipoprotein H
Friedrich-Schiller-Universität Jena

External Funding 2008/2009

Drittmittel 2008/2009

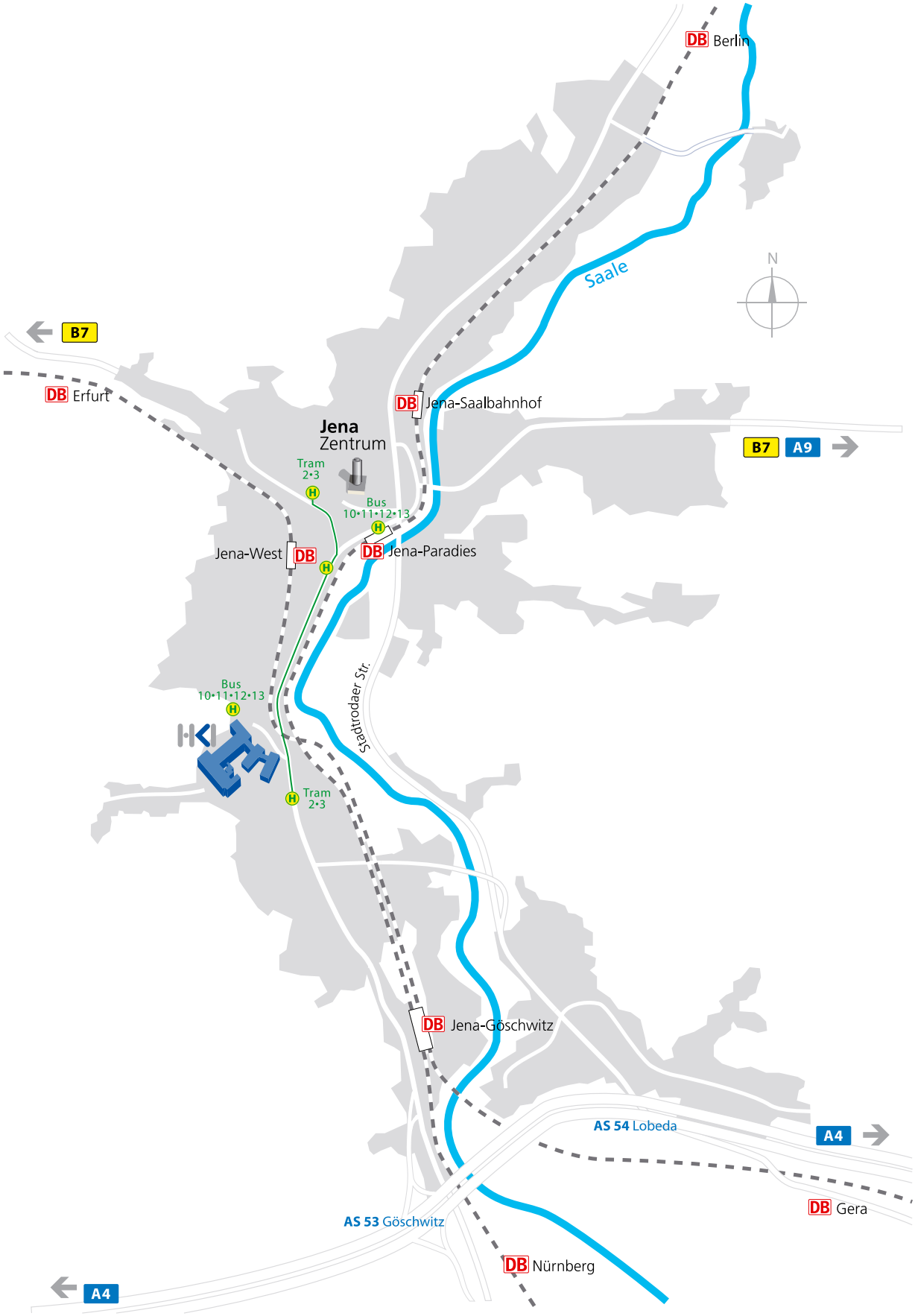


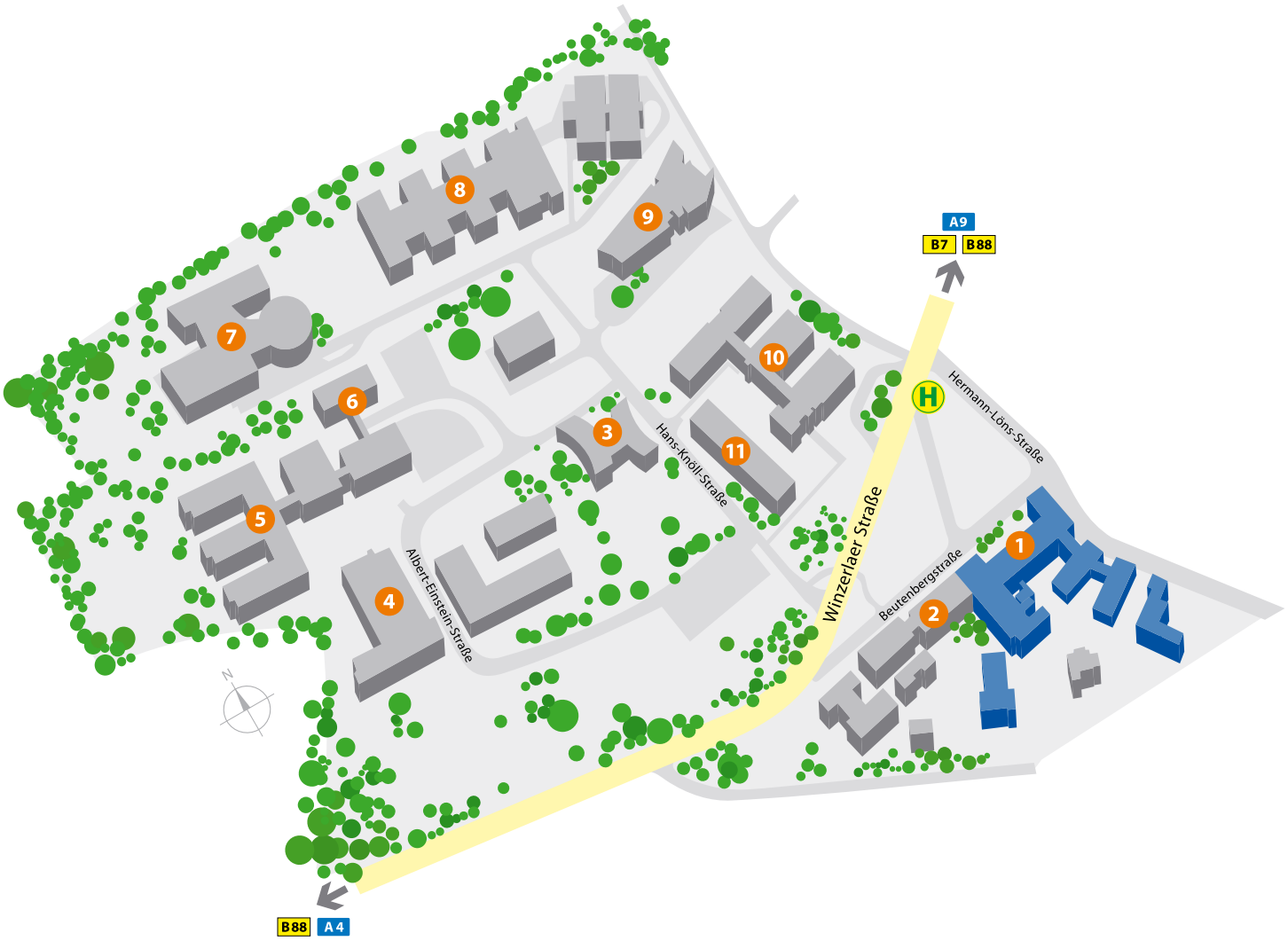


Third-party funds during the period reported: composition of the sources of income
Drittmittelausgaben im Berichtszeitraum: Verteilung über die Drittmittelgeber

Maps

Lagepläne





1. **HKI** Leibniz-Institut für Naturstoff-Forschung und Infektionsbiologie – Hans-Knöll-Institut
2. Leibniz-Institut für Altersforschung – Fritz-Lipmann-Institut
3. Abbe-Zentrum Beutenberg
4. Fraunhofer-Institut für Angewandte Optik und Feinmechanik
5. Institut für Physikalische Hochtechnologien
6. Institut für Angewandte Physik (Friedrich-Schiller-Universität Jena)
7. Max-Planck-Institut für Biogeochemie
8. Max-Planck-Institut für chemische Ökologie
9. Technologie- und Innovationspark
10. BioInstrumentezentrum
11. Centrum für Molekulare Biomedizin (Friedrich-Schiller-Universität Jena)

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