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

Leibniz-Gemeinschaft

Research Report 2010 | 2011

HKI Research Report 2010/2011

The years 2010 and 2011 at the Leibniz Institute for Natural Product Research and Infection Biology – Hans Knöll Institute were characterised by new recruitments, new developments and replacements.

As of 1 January 2011, Prof. Dr. Marc Thilo Figge took over the professorship for Applied Systems Biology at the Friedrich Schiller University Jena and became head of the research group of the same title at the HKI. Thus, Prof. Figge adds to the already existing, extensive expertise in the field of bio-informatics/systems biology at the HKI.

In addition, the Centre for Innovation Competence (ZIK) Septomics was able to win a new team member: With her appointment to the University Hospital Jena on 1 February 2011, Prof. Dr. Hortense Slevogt took over the supervision of the research group Host Septomics. In collaboration with Prof. Dr. Oliver Kurzai, who is head of the group Fungal Septomics, Prof. Slevogt, who used to be a medical practitioner at the Charité Universitätsmedizin Berlin, is now dealing with the aspect of host responses to sepsis pathogens.

We are particularly proud of the appointment of Dr. Severin Sasso to a junior professorship at the Friedrich Schiller University Jena. He qualified for an academic career on our premises with research work on natural product synthesis in algae.

The fusion of the extensive collections of micro-organisms of the HKI and the Friedrich Schiller University Jena was a milestone in the development and profiling of the HKI. 45,000 bacterial and fungal isolates are now

INTRODUCTION | VORWORT

Die Jahre 2010 und 2011 waren am Leibniz-Institut für Naturstoff-Forschung und Infektionsbiologie – Hans-Knöll-Institut von Neuzugängen, Neuentwicklungen und Neubesetzungen geprägt.

Prof. Dr. Marc Thilo Figge übernahm ab dem 1. Januar 2011 die Professur für Angewandte Systembiologie der Friedrich-Schiller-Universität Jena und damit die Leitungsposition der gleichnamigen Forschungsgruppe am HKI. Prof. Figge ergänzt damit die am HKI bereits bestehende, umfangreiche Expertise auf dem Gebiet der Bioinformatik/Systembiologie.

Außerdem konnte das Zentrum für Innovationskompetenz Septomics eine neue Mitstreiterin für die Sepsisforschung gewinnen: Prof. Dr. Hortense Slevogt übernahm mit ihrer Berufung an das Universitätsklinikum Jena am 1. Februar 2011 die Leitung der Forschungsgruppe Host Septomics. Neben Prof. Dr. Oliver Kurzai, der die Gruppe Fungal Septomics leitet, beschäftigt sich nun Prof. Dr. Slevogt, die zuvor als Medizinerin an der Charité Universitätsmedizin Berlin tätig war, mit dem Aspekt der Wirtsantwort auf Sepsiserreger.

Besonders stolz sind wir auf die Berufung von Dr. Severin Sasso auf eine Junior-Professur an die Friedrich-Schiller-Universität Jena. Er qualifizierte sich mit Forschungsarbeiten zur Naturstoffbildung in Algen in unserem Hause für eine akademische Karriere.

Ein Meilenstein in der Entwicklung und Profilierung des HKI war die Fusion der umfangreichen Mikroorganismen-Sammlungen des HKI und der Friedrich-Schiller-Universität Jena. 45.000 Bakterien- und Pilzisolate werden nun vereint in Flüssigstickstoffbehältern in den Räumlichkeiten des HKI gelagert. Sie

commonly stored in liquid nitrogen tanks on the premises of the HKI. They are an important basis for the work of micro-biologists in and outside Jena. PD Dr. Kerstin Voigt was appointed the head of the newly established Jena Microbial Resource Collection. She coordinates the global interaction with associated data bases as well as the international exchange of micro-organisms.

But also with regard to science, the years 2010 and 2011 were busy and efficient at the HKI. The head of the department Biomolecular Chemistry, Prof. Dr. Christian Hertweck, and his team discovered the first secondary metabolite from a strictly anaerobe bacterium – Closthioamid. This relates to a highly potential antibacterial component that is generated by *Clostridium cellulolyticum* that is widespread in the soil and, among others, addresses strains of the dreaded MRSA (methicillin-resistant *Staphylococcus aureus*) effectively. Furthermore, the team of Prof. Dr. Peter F. Zipfel, head of the department Infection Biology, managed to develop and apply, for the first time ever, a successful therapy against a kidney disease that had been rated incurable to date. Together with colleagues from Berlin, Heidelberg, Munster and Shanghai they initiated an immunotherapy followed by a kidney transplantation to help combatting the membranoproliferative glomerulonephritis. This scientific success received international recognition and was published in the *New England Journal of Medicine*.

The numerous further achievements of scientists at the HKI in 2010 and 2011 were published in 300 scientific works, including more than 250 original works in *peer-*

sind eine wichtige Arbeitsgrundlage für Mikrobiologen innerhalb und weit außerhalb Jenas. PD Dr. Kerstin Voigt wurde zur Leiterin der neu gegründeten Jena Microbial Resource Collection berufen. Sie koordiniert die weltweite Vernetzung der zugehörigen Datenbanken und den internationalen Austausch von Mikroorganismen.

Doch auch in wissenschaftlicher Hinsicht waren die Jahre 2010 und 2011 am HKI arbeits- und ertragsreich. So entdeckten der Leiter der Abteilung Biomolekulare Chemie, Prof. Dr. Christian Hertweck, und sein Team den ersten Sekundärmetaboliten aus einem strikt anaeroben Bakterium – Closthioamid. Es handelt sich dabei um einen hochpotenten antibakteriellen Wirkstoff, der von dem im Boden verbreiteten *Clostridium cellulolyticum* gebildet wird und unter anderem gegen Stämme des gefürchteten MRSA (Methicillin-resistenter *Staphylococcus aureus*) wirksam ist. Des Weiteren ist es einer Gruppe um Prof. Dr. Peter F. Zipfel, Leiter der Abteilung Infektionsbiologie, erstmals gelungen eine erfolgreiche Therapie für eine bislang unheilbare Nierenerkrankung zu entwickeln und anzuwenden. Gemeinsam mit Kollegen aus Berlin, Heidelberg, Münster und Shanghai setzten sie eine Immuntherapie gefolgt von einer Nierentransplantation zur Bekämpfung der membranoproliferativen Glomerulonephritis ein. Dieser Erfolg fand international große Beachtung und wurde im *New England Journal of Medicine* veröffentlicht.

Die zahlreichen weiteren Forschungsergebnisse, die die Wissenschaftler des HKI 2010 und 2011 hervorbrachten, wurden in 300 wissenschaftlichen Arbeiten veröffentlicht, davon mehr als 250 Originalarbeiten in Zeitschriften mit peer-review-System. Sie erreich-

reviewed journals. Their cumulated impact factor reached over 1,100.

Several scientists of our institute were awarded for their performances within the last two years. The founding director Prof. Dr. Wolfgang Knorre was awarded the Federal Cross of Merit for his insistent commitment at the Beutenberg Campus and the profiling of the HKI. Marcel Thön was awarded the science prize "Life Science meets Physics" of the Beutenberg Campus for his outstanding dissertation project. 15 members of staff who committed to the networking of groups at the HKI very strongly were awarded for their excellent work and honoured with the medac-research prize in the years 2010 and 2011. Special thanks go to the company medac GmbH that has been donating this prize for many years.

Research, however, can only be successful if it allows exchange not only within the institute but also beyond borders. The „Symposium on the occasion of the 25th anniversary VAAM – microbiology and society" which Prof. Dr. Axel Brakhage as the president of the Association for General and Applied Microbiology (VAAM) had invited to and which took place in the assembly hall of the Friedrich-Schiller-University Jena enjoying a long tradition, constituted a highlight in this context. Well-reputed micro-biologists from all over Germany lectured on the global, societal and economic importance of microbiology. Moreover, the doctoral researchers of the excellence graduate school *Jena School for Microbial Communication* initiated an extraordinary kind of conference: „*MiCom 2010 – European Student Conference on Microbial Communication*". That conference was par-

ten damit einen kumulierten Impact-Faktor von über 1.100.

Einige Wissenschaftler unseres Instituts erfuhren in den vergangenen zwei Jahren darüber hinaus besondere Auszeichnungen. So wurde Gründungsdirektor Prof. Dr. Wolfgang Knorre für sein unermüdliches Wirken für den Beutenberg Campus und die Profilierung des HKI mit dem Bundesverdienstkreuz geehrt. Marcel Thön erhielt für seine herausragende Doktorarbeit den Wissenschaftspreis „Life Science meets Physics" des Beutenberg Campus. 15 Mitarbeiter und Mitarbeiterinnen wurden aufgrund ihrer exzellenten Arbeiten, die zur Vernetzungen der Gruppen am HKI beitrugen, außerdem mit dem medac-Forschungspreis in den Jahren 2010 und 2011 geehrt. Ein besonderer Dank gilt dabei der Firma medac GmbH, die diesen Preis seit vielen Jahren stiftet.

Zu erfolgreicher Forschung gehört natürlich auch, dass man sich nicht nur innerhalb des Instituts, sondern auch über dessen Grenzen hinaus austauscht. Ein Höhepunkt in dieser Hinsicht war das „Festsymposium 25 Jahre VAAM – Mikrobiologie und Gesellschaft", das von Prof. Dr. Axel Brakhage als Präsident ausgerichtet wurde und in der traditionsreichen Aula der Friedrich-Schiller-Universität Jena stattfand. Namhafte Mikrobiologen aus ganz Deutschland referierten dort über die globale, gesellschaftliche und ökonomische Bedeutung der Mikrobiologie. Eine außergewöhnliche Art von Konferenz stellten überdies Doktoranden und Doktorandinnen des HKI und benachbarter Institute auf die Beine: „MiCom 2010 – European Student Conference on Microbial Communication". Diese Tagung richtet sich im Besonderen an Nachwuchswissenschaftler und begründet damit ein neues, innovatives Format der in-

ticularly aimed at junior scientists addressing a new, innovative format of international networking – "by doctoral researchers for doctoral researchers", as it was described by the initiators of the "MiCom" themselves.

The institute is constantly growing, networking and increasing. In order to allow room for growth, we lay the foundations for a modern research building on 15 September 2010. Following the recommendation that the Senate Committee on Evaluation of the Leibniz-Association provided during the evaluation procedures in 2007, not only the work surroundings for the staff at the HKI were meant to improve but the capacities for animal housing were meant to expand as well. Like this, we will take care of optimal research conditions in the future as well.

I would like to thank all colleagues at the HKI for their excellent cooperation. Only the excellent collaboration makes it possible to maintain and increase the high quality standards of scientific performance. My appreciation for the contribution to this report 2010/11 goes to all staff at the HKI, and particularly to Dr. Christine Vogler and Dr. Michael Ramm.

Jena, June 2012

Axel Brakhage
Director of the HKI

ternationalen Vernetzung – „von Doktoranden für Doktoranden", wie es die Macher der „MiCom" selbst beschreiben.

Das Institut entwickelt sich stetig weiter, vernetzt und vergrößert sich. Um diesem Wachstum Raum zu geben, legten wir am 15. September 2010 den Grundstein für ein modernes Forschungsgebäude. Mit der Umsetzung der Empfehlung des Senatsausschuss Evaluierung der Leibniz-Gemeinschaft, welche zur Evaluierung 2007 gegeben wurde, sollten sich nicht nur die räumlichen Bedingungen der Mitarbeiter und Mitarbeiterinnen verbessern, sondern auch die Kapazitäten der Tierhaltung erhöhen. Damit sorgen wir auch zukünftig für optimale Forschungsbedingungen.

Für die ausgezeichnete Kooperation möchte ich mich bei allen Kolleginnen und Kollegen des HKI herzlich bedanken. Nur durch hervorragende Zusammenarbeit ist es möglich, die hohe Qualität der wissenschaftlichen Leistung zu halten und weiter zu steigern. Für die Mitarbeit an diesem Report 2010/11 danke ich allen Mitarbeitern des HKI, insbesondere Dr. Christine Vogler und Dr. Michael Ramm.

Jena, im Juni 2012

Axel Brakhage
Direktor des HKI

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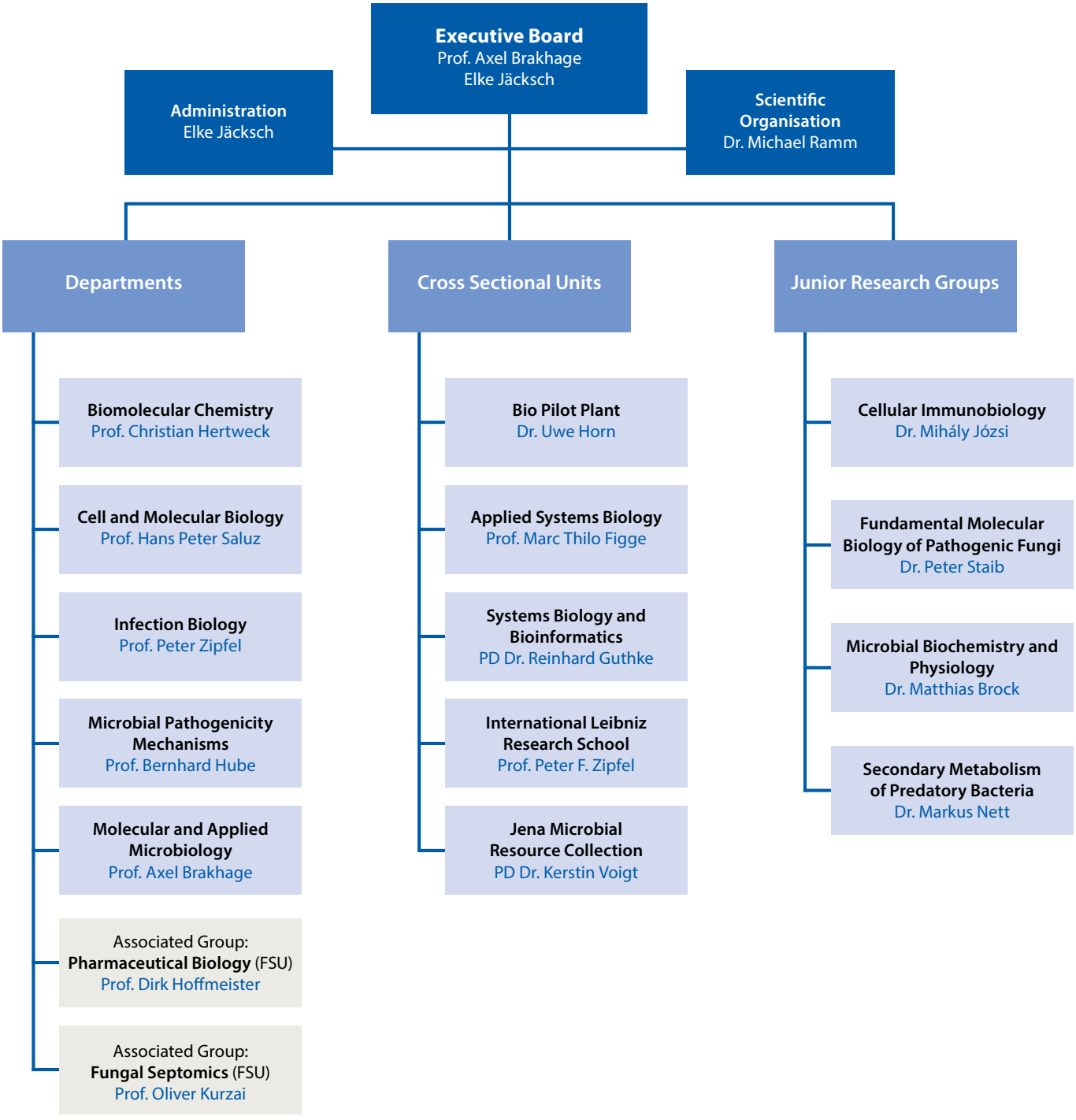
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Department of Biomolecular Chemistry

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 harbours the natural product discovery group, which is specialised 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 methods. In nature, structural and func-

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 Biosynthesewegen über biologische und

tional 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 causative agent of rice seedling blight. In the department var-

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 (*Burkholderia sp.*) und Pilzen (*Rhizopus microsporus*) bildet das antimitotisch

ious 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.

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.

1 Screening for bioactive natural products

Group Leaders:
Ling Ding, Christian Hertweck

The Department of Biomolecular Chemistry explores various resources for the discovery of novel biologically active natural products. In the last two years, our major screening programmes involved actinomycetes 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 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 various other metabolically prolific bacteria and fungi.

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 optimise 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 JMRC team and external collaboration partners (e.g. Oncotest). Our major goal is to access meaningful, and best, structurally new, compounds in the subsequent chemical projects including chromatographic isolation and structure elucidation.

Examples for new natural products discovered in the Department of Biomolecular Chemistry include Hygrobafilomycin, a cytotoxic and antifungal macrolide bearing a unique monoalkylmaleic anhydride moiety, from *Streptomyces varsoviensis*, botryorhodines A-D, antifungal and cytotoxic depsidones from *Botryosphaeria rhodina*, an endophyte of the medicinal plant *Bidens pilosa*, and farinamycin, a quinazoline from *Streptomyces griseus* (collaboration with Dr. Markus Nett, Junior Research Group Secondary Metabolism of Predatory Bacteria).

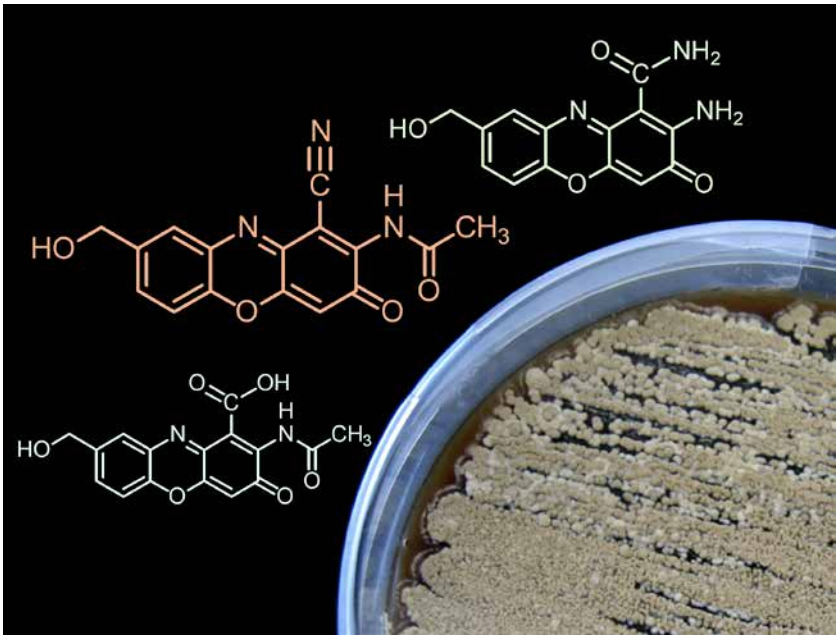


Figure 1
Bezerramycins A-C, antiproliferative phenoxazinones from *Streptomyces griseus*.

Various compounds featuring the phenoxazinone scaffold have been isolated from a *Streptomyces griseus* strain, including the antiproliferative bezerramycins A-C, chandranamycin and pitucamycin. The new bezerramycins primarily differ in their carboxy, carboxamide and nitrile substituents at C-1 (Figure 1). Natural products featuring nitrile moieties are relatively rare in nature

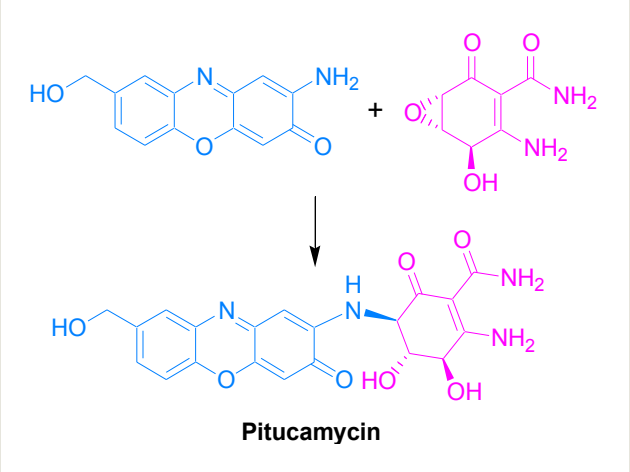


Figure 2
Pitucamycin: structural merger of a phenoxazinone with an epoxyquinone antibiotic.

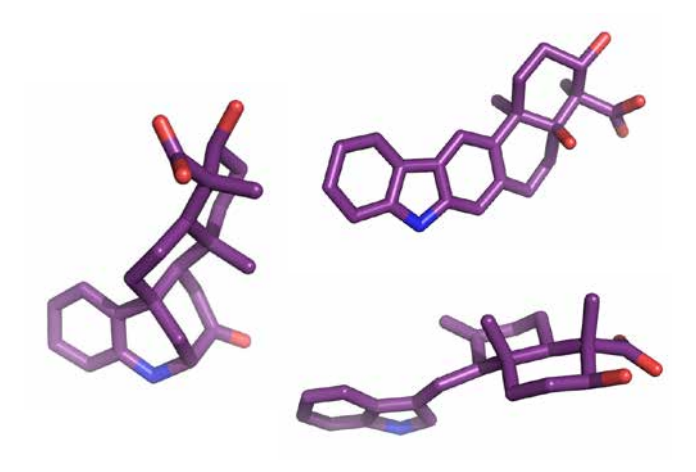


Figure 3
A family of multicyclic indolosesquiterpenes from a bacterial endophyte.

(~120 out of >170,000), and to the best of our knowledge, this is the first report of a nitrile-substituted phenoxazinone metabolite. The cooccurence of carboxy, carboxamide and nitrile substituted congeners is intriguing as it implies a biogenetic relationship. Chandranamycin D showed strong antiproliferative activities in a range of tumor cell lines (with GI_{50} values down to 1.3 μM) and is thus of interest from a pharmacological point of view, in particular with respect to the low cytotoxicity observed. We also propose the unprecedented merger of the phenoxazinone core with the amide derivative of the epoxyquinone antibiotic enaminomycin C, giving rise to the unique pitucamycin structure (Figure 2, collaboration with Dr. Markus Nett).

Indolosesquiterpenes represent a group of alkaloids with diverse biological activities, including antibiotics, antiparasitic agents, non-steroidal progestins, and inhibitors of lipid droplet biosynthesis. Interestingly, indolosesquiterpenes were primarily found in plants, and only a few examples from fungi are known. We have investigated the metabolic capability of a bacterial endophyte of the mangrove tree *Kandelia candel* and isolated several unusual multicyclic indolosesquiterpenes, xiamycins A and B, indosepene and sespenine (Figure 3). While these compounds show no activity against human tumor cell lines, in agar diffusion tests xiamycins proved to be active against several gram-positive and gram-negative bacteria, including multi-resistant ones. The newly discovered indosespene, sespenine, along with xiamycin and oridamycin, represent the first examples of indolosesquiterpenes isolated from bacteria. Furthermore, their structures and the co-occurrence of these metabolites in one strain suggest a biogenetic relationship, and a plausible biosynthetic model was established. It is intriguing that bacterial endophytes produce typical plant metabolites, and our work provides the basis

for future molecular studies to unveil any potential evolutionary relationships.

Ansa macrolides (or ansamycins) comprise a diverse group of complex, often remarkably bioactive natural products that have been predominantly isolated from actinomycetes. We have isolated and fully characterised through extensive NMR and x-ray analyses four novel ansamycins, named divergolides A-D, from an endophyte of the mangrove tree *Bruguiera gymnorrhiza* (Figure 4). Despite the significant differences in the overall architectures of the divergolides, their substitution pattern pointed to a common biosynthetic precursor. The polyketide backbone likely derives from a novel branched polyketide synthase extender unit, and is disrupted through a Baeyer-Villiger oxidation. The inherent reactivity of the polyketide precursor allows various reaction channels to yield structurally intriguing ansa macrolides. The reactive amino(hydro)quinone core sets the stage for three different core cyclizations and two final heterocyclizations, thus leading to varying macrolide ring sizes and overall topologies. Not surprisingly, these diverging pathways result in metabolites that differ in their bioactivity profiles and cover antibacterial and cytotoxic properties. To the best of our knowledge, this degree of "in-built diversification" is unprecedented for complex polyketides and highlights the beauty of biosynthetic versatility in nature.

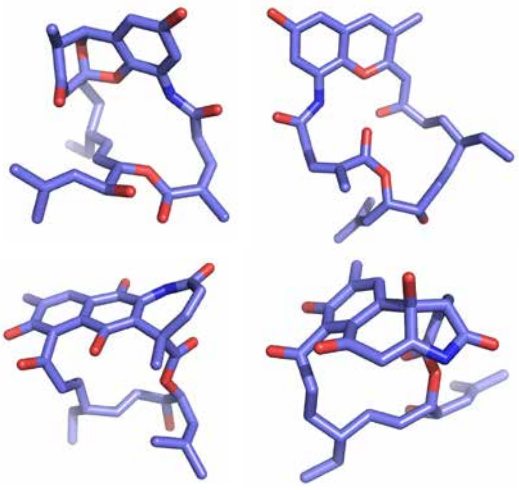


Figure 4
Divergolides A-D from a mangrove endophyte reveal an unparalleled plasticity in ansa macrolide biosynthesis.

Hydrazidomycins were another interesting discovery. Whereas synthetic hydrazines are produced in bulk quantities for industrial, agricultural and medicinal use, naturally occurring hydrazides are scarce. Three rare alkyhydrazide natural products, named hydrazidomycins A-C, were isolated from a *Streptomyces atratus* culture. Their structures, which feature a highly unusual trisubstituted hydrazine core structure, were elucidated by MS and NMR techniques (Figure 5). Depending on the substitution pattern, in particular regarding the saturation of the alkyl side chain, the hydrazidomycins exhibit moderate to strong antitumoral activities in a broad panel of cell lines, with hydrazidomycin A being the most potent compound (mean IC_{50} = 0.37 μM). Our findings not only provide an important new addition to the small family of microbial hydrazide metabolites, but also reveal a new scaffold for further development as an antitumoral therapeutic.

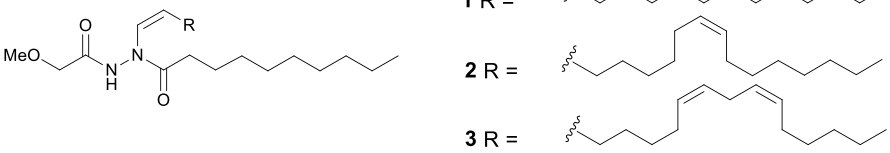


Figure 5
Structures of antitumoral hydrazidomycins.

2 Biosynthetic mechanisms and processing lines

Group Leaders:
Zhongli Xu, Christian Hertweck

In nature polyketides are generally assembled from malonyl- (MCoA) and methylmalonyl-CoA (mMCoA) units, and structural diversity is primarily governed through varying chain length, processing of the beta-keto group, versatile cyclizations, and numerous post-PKS tailoring reactions. With the aim to increase natural polyketide diversity, much effort is currently devoted to enlarging the repertoire of possible biosynthetic building blocks. We have discovered the biosynthetic origin of an unprecedented isobutylmalonyl (ibMCoA) extender unit, which is surprisingly employed in two unrelated polyketide pathways in an endophytic *Streptomyces* species. Our bioinformatic, genetic and chemi-

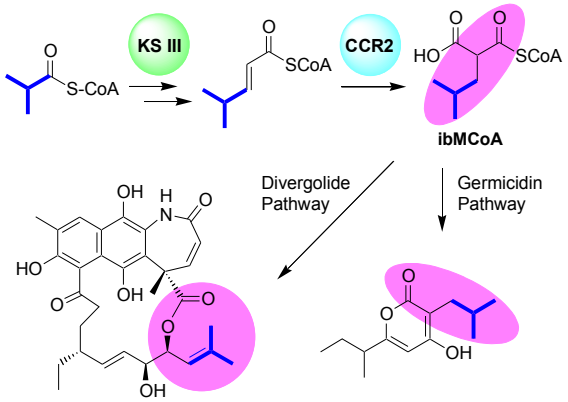


Figure 6
A branched extender unit shared between two orthogonal polyketide pathways in an endophyte.

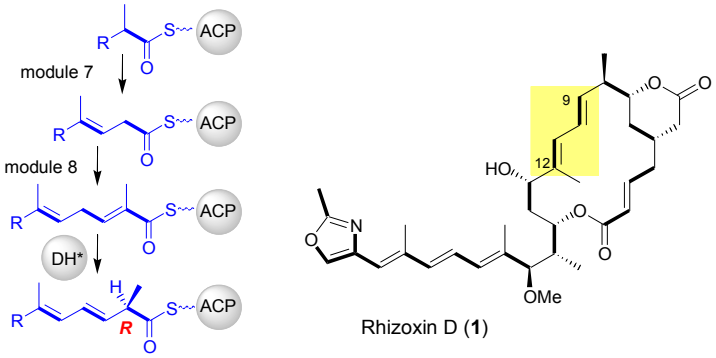


Figure 7
Double bond shifts in the biosynthesis of rhizoxin.

cal analyses not only provided new insights into the formation of the unusual branched building block, but also revealed that two fully unrelated polyketide biosynthetic pathways (a modular type I PKS and a type III PKS) share the same exotic extender unit in a wild type strain (Figure 6). To the best of our knowledge, such type of naturally occurring combinatorial biosynthesis is fully unprecedented. In this instance, the utilization of the novel extender unit by the type III PKS even leads to a change in bioactivity, that is: antibiotic activity against various bacteria. Finally, from a practical point of view, our work delivers the molecular basis for the formation of a novel PKS building block, which may be used for rationally engineering polyketide diversity.

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 *Rhizopus 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, for example in the positioning of the double bonds, which is critical for the overall spatial arrangement and biological activity of rhizoxin (Figure 7).

In both fatty acid and polyketide biosynthesis, a direct consequence of the *anti*-elimination of water from the β-hydroxy-substituted intermediate is that double bonds are generally located in-between the incorporated acetate units. However, in various metabolites double bonds are placed at atypical positions, mainly shifted from α,β- to β,γ-positions.

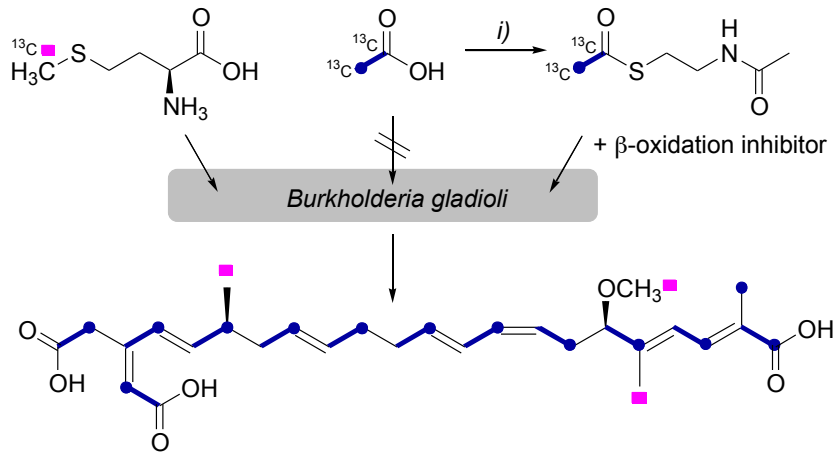


Figure 8
Biosynthetic origin of the NAD translocase inhibitor bongkrekic acid.

Such double bond migrations are a hallmark of desaturase-independent PUFA pathways, and they are also observed in a variety of complex polyketide pathways. Even so, direct evidence for the timing and the underlying biochemical operators is lacking.

We have investigated the biogenesis of the shifted diene moiety of the antimitotic macrolide rhizoxin D from the endofungal bacterium *Burkholderia rhizoxinica*. Isolation and full structure elucidation of complex biosynthetic intermediates that are released in minute amounts from a mutant blocked in the off-loading mechanism revealed the timing of desaturation and double bond migration. Analysis of two isomeric intermediates, which could only be separated through THP formation, led to the surprising discovery that the conjugated double bonds are shifted sequentially. According to the modular architecture of the *rhi* PKS, the first migration takes place concomitant with desaturation in module 7. In stark contrast, the second double bond is first generated by module 8 and then shifted by a novel type of 'shift module' featuring a noncanonical dehydratase domain (DH*). The function of the unusual DH* domain was corroborated by gene disruption and detailed analysis of the metabolites produced by the mutant. Bioinformatic analyses did not show any obvious characteristic of the DH7 domain. However, the DH* domain lacks critical motifs for dehydration and seems to be designated for α,β → β,γ-double bond shifts. The predicted course of DH*-mediated proton migration is supported by the absolute configuration of C-9 in the macrolide. These first insights into the molecular basis of double bond migration in polyketide biosynthesis are novel and fully unexpected. They may aid in the analysis of related fatty acid and polyketide synthases and provide the ground for rationally engineering novel polyene systems.

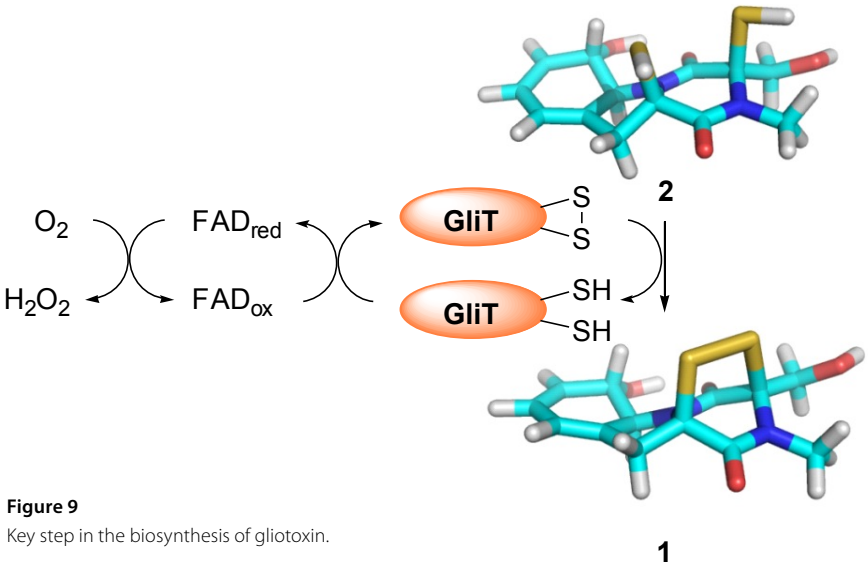


Figure 9
Key step in the biosynthesis of gliotoxin.

Bongkrekic acid is a toxin frequently occurring in Tempe Bongkrek, a popular coconut-based fermented meat surrogate. Biochemical studies revealed that bongkrekic acid efficiently inhibits oxidative phosphorylation in mitochondria through blockage of adenine nucleotide translocation, which could also be of medicinal use. Thus we aimed at unveiling the biogenetic origin of bongkrekic acid. Only through inhibiting β-oxidation in *Burkholderia gladioli* and providing synthetic (1,2-^{13}C)acetyl SNAC we succeeded in stable isotope labelling of the carbon backbone of the molecule (Figure 8). Analysis of satellite signals revealed for that bongkrekic acid is a polyketide metabolite. Furthermore, through (1^{13}C-methyl)methionine labelling and mechanistic considerations regarding α- and β-branching, we were able to deduce the direction of chain assembly. These data also led to the surprising conclusion that the C-22 carboxylate corresponds to a former acetate methylene group. Our results not only highlight the biogenetic origin of an important food-bourne toxin, but are also of importance for future molecular studies with the aim to monitor potential toxin producers.

Gliotoxin plays a critical role for virulence in invasive aspergillosis, the leading cause for death in immunocompromised patients. Gliotoxin is also the prototype of a small family of epipolythiodioxopiperazines (ETPs), which feature unique transannular di- or polysulfide bridges. Extensive molecular studies have revealed that this rare structural motif is indispensable for bioactivity and is the key to the deleterious effects of the toxin (in particular redox cycling and protein conjugation). However, to date it has remained fully enigmatic how the sulphur is incorporated into the cyclopeptide framework. In collaboration with the Department of Molecular and Applied Microbiology we have elucidated the enzymatic C–S-bond formation and disulfide

formation in gliotoxin. Through the targeted knock-outs, metabolic profiling, and *in vitro* reconstitution of GST activity we provide the first insight into the sulfurization steps in gliotoxin biosynthesis. We found that a prerequisite of gliotoxin C–S bond formation is the bis-hydroxylation oxygenation of the diketopiperazine (mediated by GliC), which produces an electrophile that is finally attacked by glutathione (mediated by GliG) – a sequence that is strikingly similar to what is observed in phase I/II detoxification reactions (Figure 9). These results are likely significant for a broad range of microorganisms as our phylogenetic analysis revealed that genes coding for GliG homologues are widespread in the genomes of ETP producers. GliG is related to theta class GSTs but appears to be the first representative of a new family of biosynthetic enzymes. Thus our work not only unveils key steps in the pathway of a virulence factor of a severe human pathogen, but also outlines to a new role of a microbial GST.

3 Combinatorial biosynthesis and biotransformations

Group Leaders: Robert Winkler, Christian Hertweck

Biosynthetic pathways leading to complex secondary metabolites frequently involve enzymes with intriguing synthetic capabilities. A closer investigation of such enzymes may lead to novel biocatalysts that could complement the currently available synthetic repertoire.

The enzymatic oxidation of amines is a widespread reaction that is mainly related to nitric oxide synthases, hormone or drug inactivation, and catabolic processes. In contrast, relatively little is known about the biosynthesis of nitro compounds in nature. AurF is an unusual monooxygenase that plays a crucial role in the formation of the rare *p*-nitrobenzoate (PNBA) building block in the biosynthesis of the antifungal and antitumoral agent aureothin in the bacterium *Streptomyces thioluteus*. Through *in vivo* and *in vitro* studies we have shown that AurF is essential and sufficient for the stepwise oxygenation of the amino group of *p*-aminobenzoate (PABA) to the corresponding nitro compound via hydroxylamino and nitroso intermediates.

Inspired by the AurF crystal structure and modelling experiments, we have generated an AurF mutant with an enlarged cavity at the active site, which resulted in a fourfold increase in substrate turnover (Figure 10). To invert the molecular recognition, we created a mutant in which arginine at position 96 was replaced by glutamate. This *N*-oxygenase variant proved to be incapable of transforming PABA, but accepted the guanidinyl analogue as a substrate. Interestingly, both the R96E mutant and the native enzyme accept guanidinyl- and benzamidinyl-substituted anilines as substrates, thus exerting an inverse reactivity pattern to NO-synthases. Further control experiments with alternative substrates support a new and refined model for substrate binding. Understanding the molecular basis of the chemo- and regio-selectivity of enzymatic nitro formation could aid in developing biotechnological applications of this unusual monooxygenase.

Based on structure comparisons of AurF with ribonucleotide reductases (RNRs) and through hypothesis-driven mutagenesis experiments we were able to identify an electron transfer system via hydrogen bridges in AurF that is analogous to the one found in RNRs and fatty acid desaturases (Figure 11). Furthermore, we provide strong evidence that an electron transfer chain with ferredoxin is involved in the natural activity of AurF. The growth phase dependent activity of the *N*-oxygenase and the structural similarity of AurF, RNRs and desaturases strongly suggest that electron transfer chain components are recruited from the latter. So far this is the best explanation for both the lack of accessory genes in the *aur* gene locus and the successful *in vivo* reconstitution of AurF

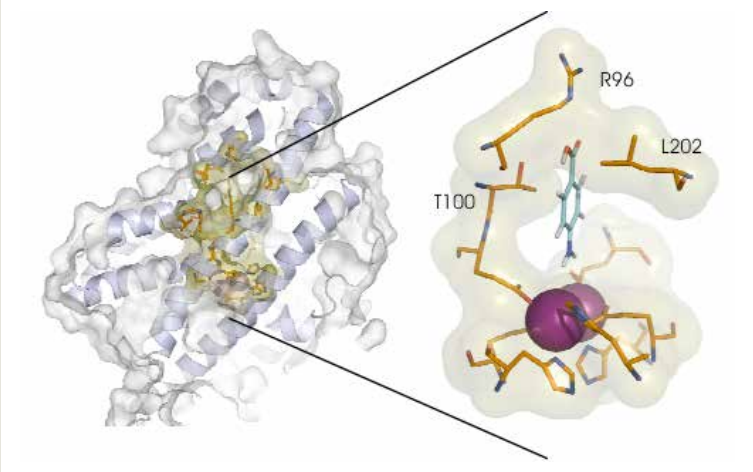


Figure 10
Model of one AurF monomer, with substrate binding pocket highlighted (left). Enlargement of the active site with bound substrate PABA and dimetal cluster (right).

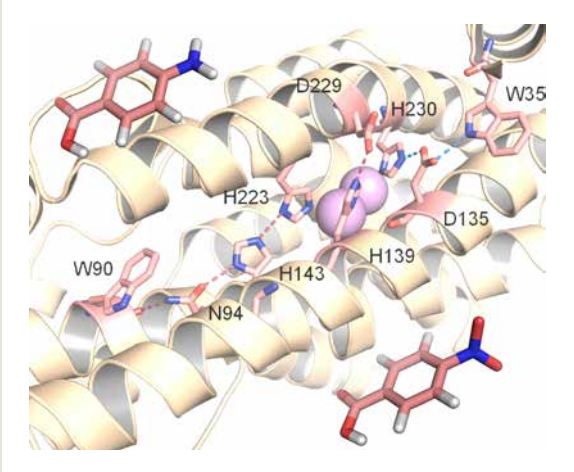


Figure 11
The unusual nitro-synthase AurF docks to ferredoxin and employs an electron transfer system using hydrogen bridges in analogy to ribonucleotide reductases (RNRs).

in different expression hosts. Thus, our findings not only have implications for biotechnological applications of this biocatalyst, but also unveil a fascinating correspondence between the electron transfer in a nitro-group forming enzyme and a ribonucleotide reductase.

Cytochrome P450 enzymes are involved in a vast number of biosynthetic pathways. A hallmark of these biocatalysts is their high regio- and stereoselectivity, even when modifying non-activated C-H groups. Because these reactions are particularly difficult or even impossible to emulate with the currently available synthetic protocols, P450s have become particularly valuable for biotechnological applications, in particular as biocatalysts. AurH is a unique cytochrome P450 monooxygenase catalyzing the stepwise formation of a homochiral oxygen heterocycle, a key structural and pharmacophoric component of the antibiotic aureothin. The exceptional enzymatic reaction involves a tandem oxygenation process including a regio- and stereospecific hydroxylation, followed by heterocyclization. To unveil the biochemical basis for this unparalleled enzyme action, we have determined the crystal structure of AurH, which represent the first structures of members of the CYP151A family, in different conformational states as well as of a complex of AurH bound to the cytochrome P450 inhibitor ancymidol (Figure 12). In combination with computational docking, inhibitor studies and mutagenesis experiments, we identified the key residues for catalysis and find that the subsequent oxidation and heterocycle formation is achieved by a switch function in recognizing two substrates, deoxyaureothin and the hydroxylated intermediate. A full structure determination of three new aureothin analogues produced by AurH variants supports our model of catalysis and revealed the possibility to redirect the regio- and chemoselectivity of AurH with a single

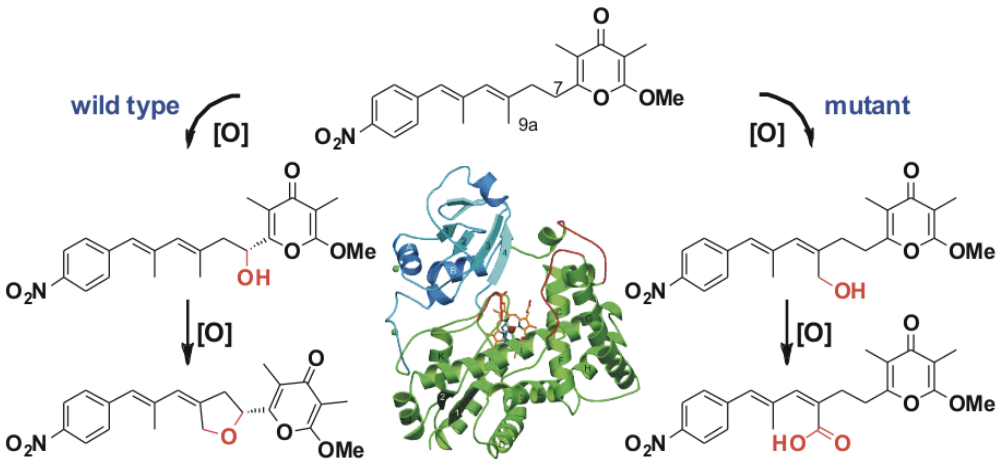


Figure 12
Structural fine-tuning of a multifunctional cytochrome P450 monooxygenase.

point mutation in the active site to catalise a regioselective six-electron transfer of a non-activated methyl group via hydroxyl and aldehyde to a carboxylic acid. Our findings shed new light on this major class of biocatalysts and demonstrate that cytochrome P450 enzymes may be fine-tuned to mediate highly specific multistep reactions.

Aureothin is a densely functionalised natural product with intriguing biological activities, yet toxic and pharmacologically unfavorable due to the nitroaryl substitutent. On the basis of the knowledge gained on the biosynthetic mechanism, we aimed at generating a series of aureothin analogues for structureactivity relationship (SAR) studies. Because of the relatively small size of both molecule and the corresponding biosynthesis gene cluster, this target was ideally suited as a model for combining genetic, biochemical and chemical methods to generate structural diversity. Furthermore, the aureothin pathway exhibits three principal levels that could be envisaged for diversification: the aryl starter unit, the oxygenated backbone, and the pyrone head group. In order to efficiently replace the nitro substituent we chose the concept of bioisosterism that is well known in drug design. This goal was achieved through mutasynthesis using an engineered Δ*aurF/aurH* null mutant that exhibits a broad substrate tolerance towards ten alternative PKS starter units. Furthermore, in a combinatorial biosynthesis approach, we found that the endogenous γ-regiospecific pyrone methyltransferase (AurI) can be replaced by an α-regiospecific homologue from the enterocin pathway (EncK), yielding the corresponding iso-deoxyaureothin derivatives (Figure 13). Finally, the aureothin cytochrome P450 monooxygenase, AurH, was used as a tool for whole cell enzymatic hydroxylation and heterocyclization, repectively, providing a convenient access to the chiral tetrahydrofuran moiety. By this highly integrative approach in total

fifteen aureothin analogues were generated on a preparative scale. A biological evaluation of these compounds revealed new insights into structure-activity relationships. Some of the new deoxyaureothin derivatives are less cytotoxic but stronger antiproliferative agents than the parent natural product. Furthermore, some of the new aureothin derivatives hold promise as highly selective antifungal, in particular anti-*Candida*, and it seems that the THF ring increases antifungal activity. Our results demonstrate that exploiting enzymatic promiscuity may well complement synthetic approaches to generate structural diversity. Thus, our results are another illustration for the viability and potential of biosynthetic engineering.

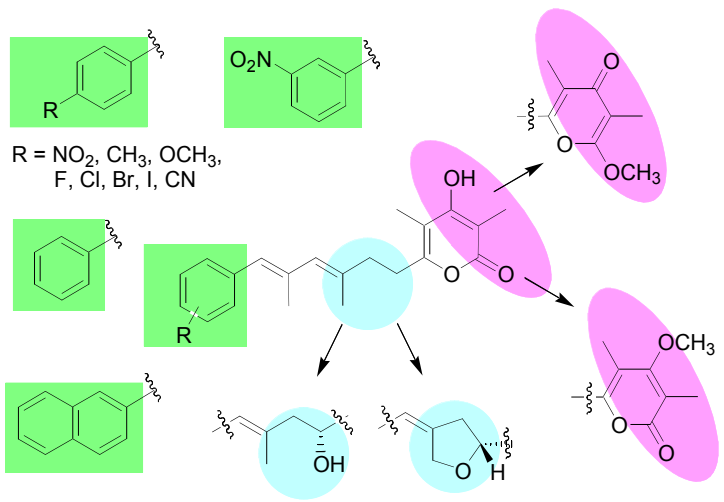


Figure 13
Combinatorial biosynthesis of antifungal and antiproliferative aureothin derivatives.

The 16-membered macrolide rhizoxin is a highly potent antiproliferative agent that destabilises microtubules by binding to the β-tubulin subunit and thus inhibits mitosis. Rhizoxin was evaluated in phase I and II clinical trials, but rapid elimination and low activity hampered its further development. To investigate structure-activity relationships we have synthesised and evaluated the antiproliferative and cytotoxic activities of 16 ester and amide functionalised rhizoxin derivatives (Figure 14). All esters display antiproliferative and cytotstatic activities at nanomolar concentrations and thus are highly potent antimitotic agents. The flexibility of the alcohol part makes the ester functionality an interesting target for drug linker approaches. Except for a primary amide, all amides are less active than the ester derivatives, which is in agreement with the results of the antifungal tests. In addition, new insights into the rhizoxin binding pocket can be deduced from the finding that the glycerol ester exhibits effective concentrations in the nanomolar range. According to calculated log K_{OW} values the glycerol ester shows the best water solubility of all derivatives tested in this study.

Oxazole and thiazole moieties as in rhizoxin are key structural features of many natural products, yet the tolerance of a PKS-NRPS hybrid towards synthetic oxazole bioisosters has not yet been investigated. We reported a systematic evaluation of an engineered PKS-NRPS assembly line towards oxazole mimics, which not only provided first insights into selection rules, but also allowed for the preparation of novel rhizoxin derivatives (Figure 15).

4 Genome mining for natural product discovery

Group Leaders: Kirstin Scherlach, Keishi Ishida, Christian Hertweck

In the postgenomic era it has become increasingly obvious that the biosynthetic potential of microorganisms has been greatly underexplored. In many organisms the majority of genes potentially coding for the biosynthesis of biologically active compounds remain silent, and new methods are required to activate such 'cryptic natural products' to light. Our aim is to develop new ways to specifically activate such cryptic gene clusters. Manipulation of the promotor for the quorum sensing regulator gene in the model organism *Burkholderia thailandensis* caused a significant change in the metabolic profile (Figure 16). This genetic manipulation led to the activation of the thailandamide biosynthesis gene cluster, and dramatically increased production of the PKS-NRPS hybrid metabolite thailandamide. In addition, a novel polyketide metabolite, thailandamide lactone, which cannot be detected in the

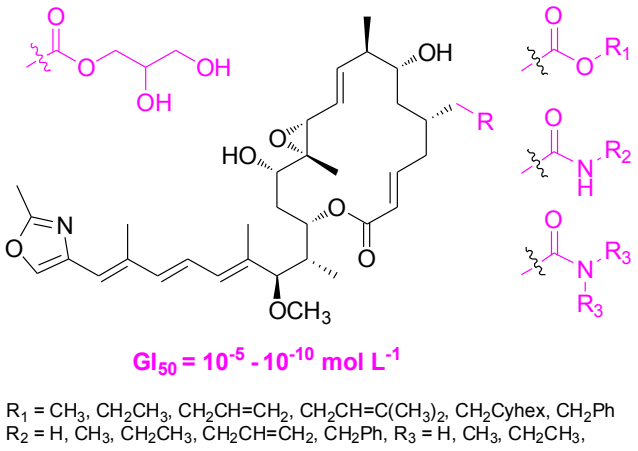


Figure 14
Semisynthesis of a set of 16 ester and amide analogues of the antimitotic macrolide rhizoxin.

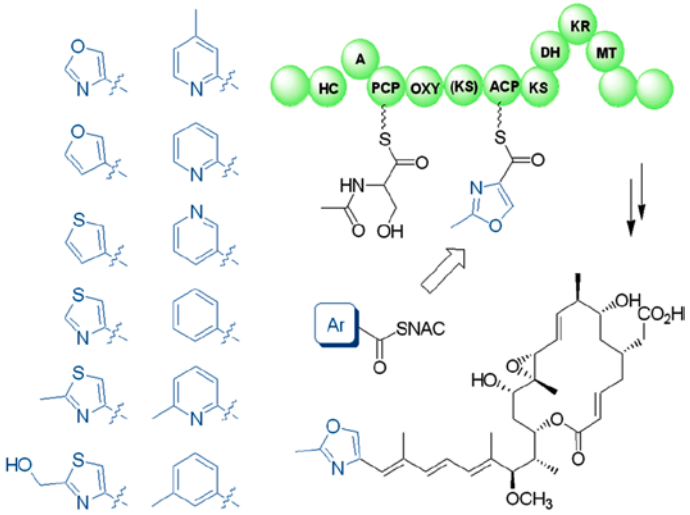


Figure 15
Mutasynthesis of rhizoxin analogues.

wild type, was isolated and its structure was elucidated by HRMS, IR and NMR. In a biological assay using tumor cell lines, the lactone showed moderate antiproliferative activities. This finding not only implies that ThaA is a rare negative quorum sensing regulator, but also serves as a proof of concept that engineering quorum sensing deletion mutants may enable the discovery of novel bioactive natural products encoded by silent or significantly downregulated biosynthetic pathways.

Bacteria belonging to the genus *Clostridium* represent some of the most prominent microorganisms that lead an obligate anaerobic lifestyle. Surprisingly, apart from protein toxins, no secondary metabolites have been isolated from these or any other strictly anaerobic bacteria. However, bioinformatics analysis of the recently sequenced genomes of *Clostridium* spp., e.g. *Clostridium kluyveri* indicates that these bacteria indeed harbour genes for the biosynthesis of secondary metabolites, which have been overlooked so far. It appears that the biosynthesis genes in anaerobes remain dormant under standard laboratory conditions and are only triggered in the presence of particular stimuli. We have tested a variety of cultivation parameters and supplements in order to stimulate secondary metabolite production. By means of aqueous soil extracts we eventually succeeded in the induction of a cryptic pathway. A hitherto fully unprecedented type of symmetrical natural product was isolated, in which all building blocks are connected through thioamide linkages (Figure 17). Thioamide moieties are frequently employed in synthetic medicinal

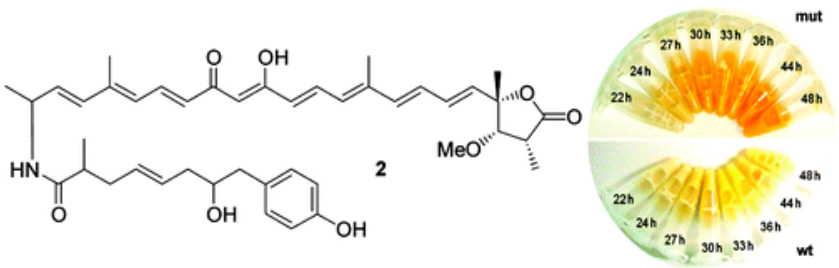


Figure 16
Induced biosynthesis of cryptic polyketide metabolites in a *Burkholderia thailandensis* quorum sensing mutant.

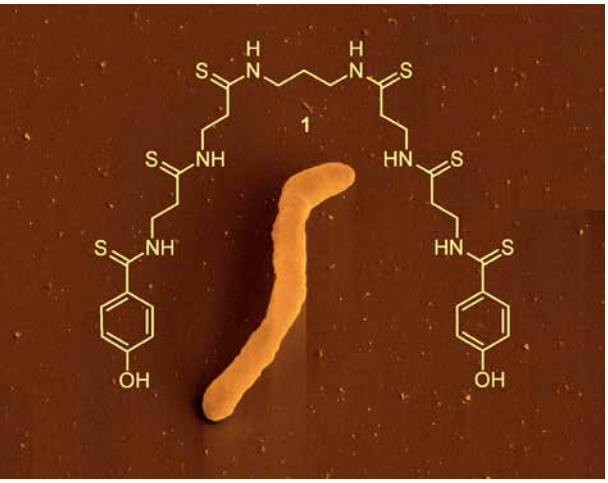
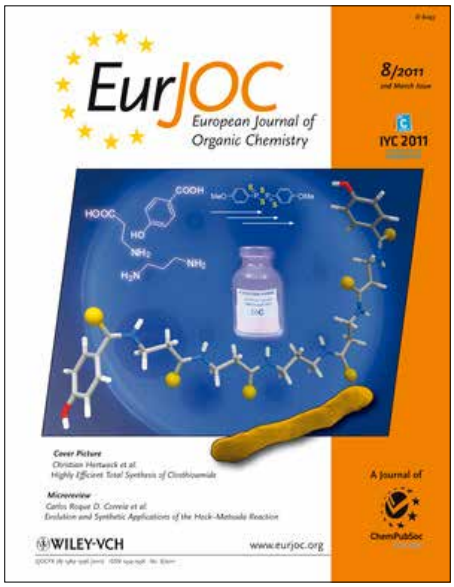


Figure 17
Micrograph of *Clostridium cellulolyticum* and structure of closthioamide, the first antibiotic from an anaerobic microorganism.

Figure 18
Cover picture of the European Journal of Organic Chemistry showing the structure of closthioamide, the first example for secondary metabolites produced by anaerobic bacteria (see bottom part, scanning electron micrograph of the natural producer *Clostridium cellulolyticum*). The natural product exhibits high antimicrobial activity, in particular against resistant pathogens (MRSA and VRE, see background). Since closthioamide represents a novel drug scaffold and its availability is limited by low production rates in *C. cellulolyticum* cultures, we developed a highly efficient total synthesis involving convergent peptide coupling and polythionation. We thank Dr. S. Nietzsche (Electron Microscopy Centre of the University Hospital, Jena) for providing scanning electron micrographs and Nico Ueberschaar for graphical processing.



chemistry and serve as isosters for amide bonds, lending higher rigidity and stability towards proteases. In stark contrast, thioamides are extremely scarce among naturally occurring organic compounds: Only four out of an estimated number of 170,000 known natural products feature thioamide groups; it should be highlighted that an entirely polythioamidated metabolite like closthioamide is totally unprecedented. This finding is significant because this is the first report on a secondary metabolite from a strictly anaerobic bacterium. Furthermore, we found that closthioamide represents a novel type of antibiotic that is highly active, even against methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococcus faecalis* (VRE), with only moderate cytotoxicity. The importance of the thioamide moieties for antibiotic activity was corroborated through the synthesis of a hexaoxa analogue (closamide), which is fully inactive against the test strains. This finding is very encouraging to delve deeper into the underexplored metabolome of anaerobes.

While closthioamide is a promising new antibacterial agent, its availability is limited by the low production rates in *C. cellulolyticum* cultures. To provide convenient access to ample amounts of the antibiotic, we have developed an efficient synthetic strategy starting from readily available starting material. The convergent synthetic route not only allows the multigram synthesis of the unique polythioamide antibiotic involving a one-pot hexathionation, but also provides the basis for a huge variety of prospective modifications. Bioactivity tests have confirmed the impressive activity of closthioamide against multiresistant bacterial pathogens. Furthermore, they revealed that the activity of the polythioamides depends on the nature of the phenyl substituents, and holds promise that their selectivity is tunable to some degree. Our protocol provides efficient access to a

variety of closthioamides and thus sets the stage for studying their mode of action and structure activity relationships – important prerequisites for the further development of this novel class of antibiotics.

Fungal genomes bear a largely untapped biosynthetic potential, impressively documented by the occurrence of a large number of cryptic or silent biosynthesis gene clusters. In eukaryotes, it is particularly challenging to activate such silent genes, and much effort is currently devoted to this area in collaboration with the Department of Molecular and Applied Microbiology. By combining biotechnological, analytical and genomic methods and tools, we discovered two novel complex polyketide metabolites from *Aspergillus nidulans*, which show interesting pharmacological features. Expression studies and comparative analyses using a knock-out strain indicate that the novel spiroanthrones formed are hybrid molecules that emerge from two different polyketide biosynthetic pathways (Figure 19). Our results not only add to the pool of available chemical structures from this model fungus. They also demonstrate for the first time that nutrient depletion using a chemostat may be an effective method to induce biogenetic pathways that were previously regarded as silent under standard conditions. The variation of culture conditions led to the discovery of as to yet unknown prenylated isoindolinone alkaloids from *A. nidulans*, named aspernidine A and B.

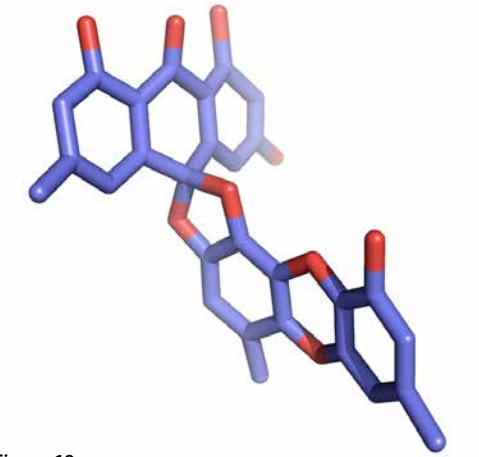


Figure 19
Structure of an unusual spiroanthone from *A. nidulans*.

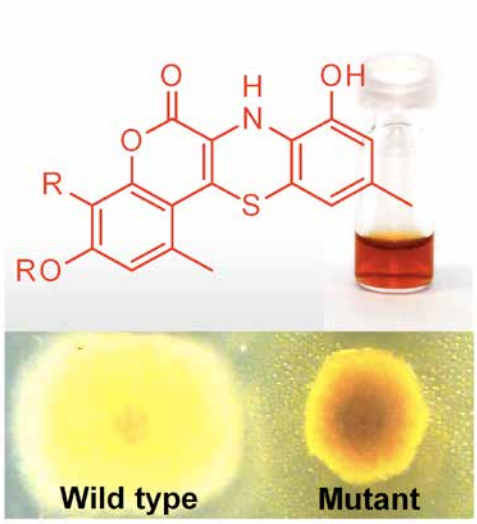


Figure 20
Formation of unprecedented red hair-like pigments in a fungus impaired in protein acetylation.

Also in collaboration with the Department of Molecular and Applied Microbiology we explored a new approach to trigger metabolite production by deleting a gene encoding the *N*-acetyltransferase NnaB in *Aspergillus nidulans*. A metabolic switch was thus induced, which allowed the isolation and structural elucidation of unparalleled fungal metabolites, named pheofungins A-D (Figure 20). We provided a body of evidence that these red pigments are the fungal response to global stress caused by impaired posttranslational modification. The structures of the pheofungins represent benzopyranobenzothiazinones that are fully unprecedented for fungi. However, the pheofungin chromophore is remarkably similar to pheomelanins, the red pigments in human hair of Celtic origin. Transcription analysis and gene knock-out experiments indicated that pheofungins result from the condensation of orsellinic acid derived phenols with cysteine in analogy to the pathway for red hair pigments. From a pharmacological point of view, the discovery of the novel benzopyranobenzothiazinones is significant because of the potent antiproliferative activity of pheofungin C. This study thus not only describes a new avenue to structurally intriguing metabolites but also illustrates that modulation of fungal regulatory systems can promote the discovery of natural products with potential therapeutic application.

Other projects in the area of genome mining include the multifactorial induction of an

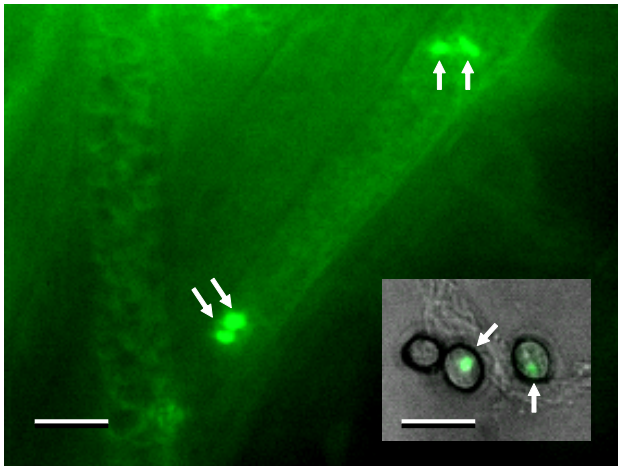


Figure 21
Confocal laser scanning micrographs of rhizoxin-producing bacterial endosymbionts (*Burkholderia rhizoxinica*, labeled with GFP) in the mycelium of *Rhizopus microsporus* (main image) and in spores (inset). Bars: 10 mm (main image), 5 mm (inset).

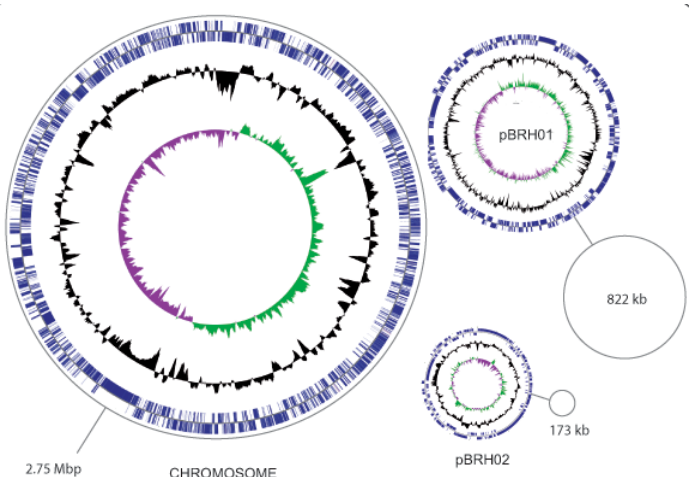


Figure 22
Genome structure of *Burkholderia rhizoxinica*, the endosymbiont of the rice seedling blight fungus *Rhizopus microsporus*.

orphan PKS-NRPS gene cluster in *Aspergillus terreus* (collaboration with Dr. Matthias Brock, Junior Research Group Microbial Biochemistry and Physiology). In collaboration with Prof. Elke Dittmann, Potsdam, we exploited the natural diversity of microviridin gene clusters for discovery of novel tricyclic depsipeptides.

5 Microbial interactions: Investigations of endofungal bacteria

Group Leaders: Gerald Lackner, Christian Hertweck

Many bacterial species colonise niches within eukaryotic host organisms as endosymbionts. Excellent studies of bacterial mutualists both of plants and animals have been described on genomic level in detail. Recent publications of symbiont genomes include symbionts of host species ranging from plants to marine microalgae and amoebae. However, symbionts of fungal species have long been a widely neglected area of research, although they have important implications for agriculture, biotechnology and food safety.

We have recently discovered an unparalleled phytopathogenic alliance of the fungus *Rhizopus microsporus*, also known as the rice seedling blight fungus, and endobacteria of the genus *Burkholderia* (Figure 21). This is the first reported case in which a fungus harbours "endofungal bacteria" for the production of a virulence factor. Very surprisingly, only in the presence of the toxin-producing endobacteria the host fungus is capable of sporulation, thus warranting the survival and distribution of the symbiosis.

To elucidate the symbiosis factors we have sequenced and analysed the whole genome of the toxin producing bacterial endosym-

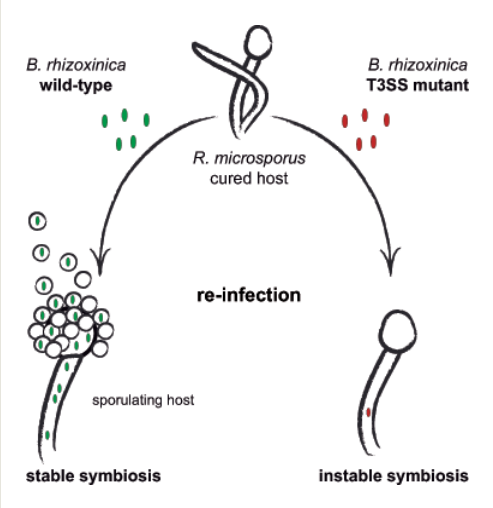


Figure 23
Impact of the bacterial type 3 secretion system on the fungal-bacterial symbiosis.

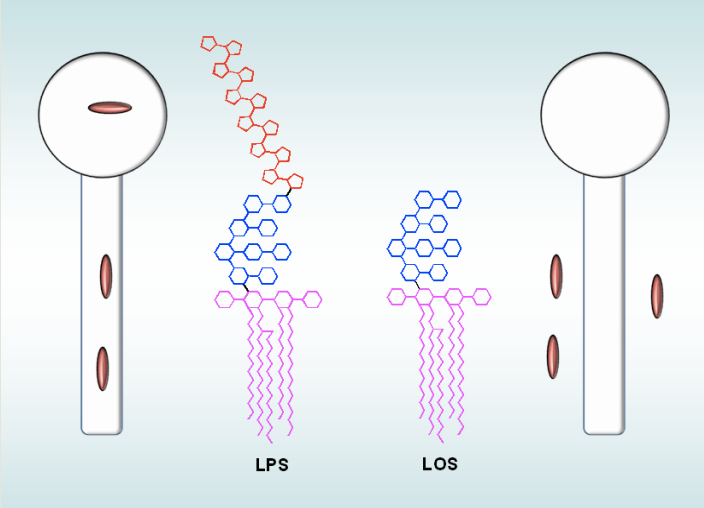


Figure 24
A lipopolysaccharide renders the endosymbiont in "stealth mode".

biont (*Burkholderia rhizoxinica*) of the rice seedling blight fungus *Rhizopus microsporus*. This is the first reported genome sequence of a mutualistic *Burkholderia* sp., and the first one of a bacterial endosymbiont of a fungus. The complete genome sequence provides unprecedented insights into the molecular equipment of a specialised symbiont that plays a key role in a tripartite microbial interaction.

B. rhizoxinica is the first endofungal bacterium, whose genome has been sequenced. We deduced models of evolution, metabolism and tools for host-symbiont interaction of the endofungal bacterium from the whole genome analyses (Figure 22). Genome size and structure suggest that *B. rhizoxinica* is in an early phase of adaptation to the intracellular lifestyle (genome in transition). By analysis of tranporters and metabolic pathways we predict how metabolites might be exchanged between the symbiont and its host. Gene clusters for biosynthesis of secondary metabolites represent novel targets for genomic mining of cryptic natural products. *In silico* analyses of virulence-associated genes, secreted proteins and effectors might inspire future studies on molecular mechanisms underlying bacterial-fungal interaction.

Through genetic studies we have shown that a type III secretion system (T3SS) encoded in the genome of the endofungal bacteria plays a crucial role in the maintenance and persistence of the bacterial-fungal alliance (Figure 23). We found that mutants defective in type III secretion show reduced intracellular survival and fail to elicit sporulation of the host. Furthermore, genes coding for T3SS components are upregulated during cocultivation of the bacterial symbiont with the host, highlighting a specific interaction between both partners. This is the first report on a T3SS involved in bacterial-fungal sym-

biosis. Furthermore, phylogenetic analysis revealed that the symbiont T3SS is distant from homologs of animal/human pathogens and represents a prototype of a clade of yet uncharacterised T3SSs within the *hrp* superfamily of T3SSs from plant pathogenic microorganisms. Thus, the finding of the first T3SS in a bacterial-fungal mutualism closes an important gap in the large body of knowledge on T3SS in symbioses.

Another mystery of the fungal-bacterial symbiosis was how the endobacteria can evade the fungal defense mechanisms and how the symbiotic partners interact by ways of chemical recognition and communication. In collaboration with Prof. Antonio Molinari (Italy) we disclose that a novel lipopolysaccharide (LPS) from the bacterial symbiont is a critical molecular determinant for the stability of the symbiosis. We have fully elucidated the first LPS structure of a bacterium living within a fungus, and revealed the presence of a unique 1,2-β-D-galactofuranose glycoconjugate. To gain insight into the biological function of the O-antigen, we have sequenced an LPS biosynthesis gene locus, which fully supports the structure elucidation. Furthermore, we succeeded in generating a targeted O-antigen ligase mutant, which is incapable of producing the poly-galactofuranoside conjugate and confirmed the absence of the O-antigenic chain by chemical analyses. A sporulation assay and microscopic investigation finally revealed that intracellular survival of the mutant is critically impaired and that the O-antigen is a crucial component for establishing a stable bacterial-fungal symbiosis (Figure 24). Considering that galactofuranose units are particularly abundant in filamentous fungi, one may conclude that the galactofuranosyl O-antigen serves as mimicry to put the bacterium into "stealth mode". To our knowledge, this is the first study shedding light on the role of surface carbohydrates in an interaction between endobacteria and fungi. Our results thus not only unveil a new complex biomolecule, but also disclose the role of a glycoconjugate in a novel biological context.

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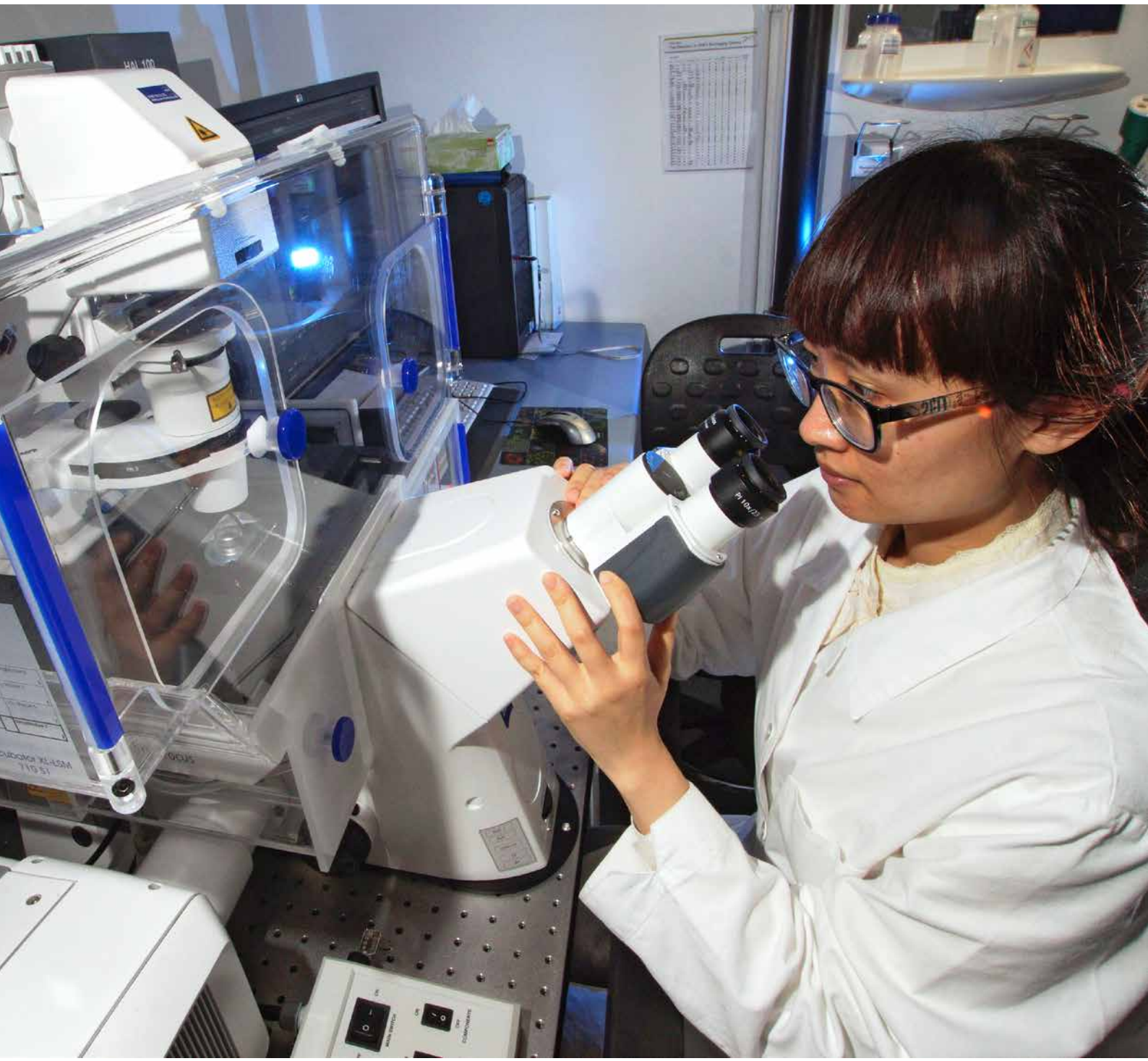

Department of Infection Biology

Department of Infection Biology

The Department of Infection Biology is interested in understanding the homeostatic balance of innate immunity that recognises and eliminates invading microorganisms and protects self tissue. Any disturbances in this tightly balanced system results in inflammation and in diseases. Imbalance is caused by pathogenic microbes, which in order to survive camouflage their surface and mimic self, and which in consequence are not properly recognised by these efficient host defence systems. Inappropriate protection of self is caused by multiple modifications including variations of self components, single molecules and proteins due to genetic causes such as sequence variations, mutations, genetic modifications, duplications or deletions of chromosomal segments. These changes disturb the homeostatic balance and can progress to inflammation, chronic reactions and ultimately to autoimmune diseases. The understanding of these processes in molecular terms allows to describe the infection process in detail, to define disease mechanisms in molecular terms and to translate the findings into new therapies.

INTRODUCTION | EINLEITUNG

Head:
Prof. Dr. Peter F. Zipfel

Die Abteilung Infektionsbiologie beschäftigt sich mit dem homöostatischem Gleichgewicht der angeborenen Immunität bei der Erkennung und Eliminierung von infektiösen Erregern und dem Schutz von körpereigenem Gewebe. Jede wesentliche Veränderung dieses homöostatischen Gleichgewichtes führt zur Erkrankung. Ungleichgewichte werden durch pathogene Erreger verursacht, die ihre Oberfläche so verändern, dass sie für das Immunsystem des Wirtes nicht erkennbar sind. So können diese Erreger in einem immunkompetenten Wirt überleben und eine Infektion hervorrufen. Genetische Sequenzveränderungen und Mutationen von einzelnen Komponenten des angeborenen Immunsystems, die zu veränderten Proteinen und Molekülen oder zu deren Verlust führen, resultieren in einem unzureichenden Schutz körpereigner Oberflächen. Die Immunreaktion wird nicht richtig kontrolliert, es kommt zu einer verstärkten Entzündungsreaktion und letztendlich zur Erkrankungen. Das genaue Verständnis dieser Prozesse ist zum einen wichtig, um den Infektionsprozess von Krankheitserregern genau zu beschreiben, zum anderen wird es benötigt, um die Mechanismen von Autoimmunerkrankungen auf molekularer Ebene zu verstehen. Diese Erkenntnisse und Konzepte werden genutzt, um neue Wege für die erfolgreiche Behandlung von Krankheiten zu finden und anzuwenden.

1 Innate immunity: The role of complement for fungal evasion

Group Leader: Peter F. Zipfel

The immune battle between *C. albicans* and the human host

Candida albicans is a medically important human fungus that can cause a wide range of disorders from superficial infections to dissemination diseases. Despite currently applied antifungal therapies, both mortality and morbidity mediated by this human pathogen are still unacceptably high. As new prophylactic and therapeutic strategies are urgently needed to prevent fungal infection, there is a need to understand the immune evasion strategies of the fungal pathogen *C. albicans* and to identify new fungal targets for combating fungal infection. Similar to other pathogenic fungi and pathogenic microbes *Candida* uses a large variety of immune evasion strategies to combat host innate and adaptive immune response. Understanding the cellular and molecular mechanisms that *C. albicans* uses in order to survive and propagate in the human host is central to understanding fungal pathogenicity. Such an understanding of the infection process in molecular terms will define new diagnostic tools and will lead to new strategies to fight and interfere with *Candida* infections.

Host protection and innate immunity:

The human host responds to fungal infections and normally uses a highly efficient immune defence system. Complement is the central part of the host innate immunity and this powerful the system is aimed to clear invading microbes and balance homeostasis. Pathogenic microbes, like *Candida albicans*, have to cross this efficient and important immune defence layer in order to propagate within the host and to establish an infection. Knowing how exactly the activated complement cascade responds to and attacks microbial invaders is central to understand the immune battle in the infection process. This also allows to define how *Candida* counteracts the individual steps of the host innate immune defence. Ultimately this knowledge will allow to design adequate therapeutic molecules.

The activated complement cascade forms several effector products, which are aimed at attacking an invading microbe. These activation fragments include C3a which displays antifungal as well as antimicrobial activity, and C3b which decorates foreign surfaces and whose generation is enhanced by an amplification loop. This opsonization by C3b enhances adhesion and phagocytosis by human immune effector cells such as macrophages and neutrophils. In addition, the C5a activation fragment induces an inflammatory circuit and activates peripheral blood mononuclear cells which in response secrete inflammatory cytokines, including TNF-a, Il-1b and IL-6. Furthermore, the terminal complement complex (TCC) forms a pore and damages membranes (Figure 1).

Chen et al. showed for the first time that C5a activates PBMCs and induces the release of inflammatory IL-6 and IL-1b which induces a cellular inflammatory response against the fungal pathogen. The group also characterised how *Candida* blocks this inflammatory cytokine response at least partially by expressing and releasing the virulence factor Pra1.

The immune battle between *C. albicans* and the human host: *C. albicans* similar to other pathogens is well prepared to control the challenge by the immune systems of its vertebrate hosts. *C. albicans* as a fungal pathogen expresses proteins that interact with host immune proteins and in conse-

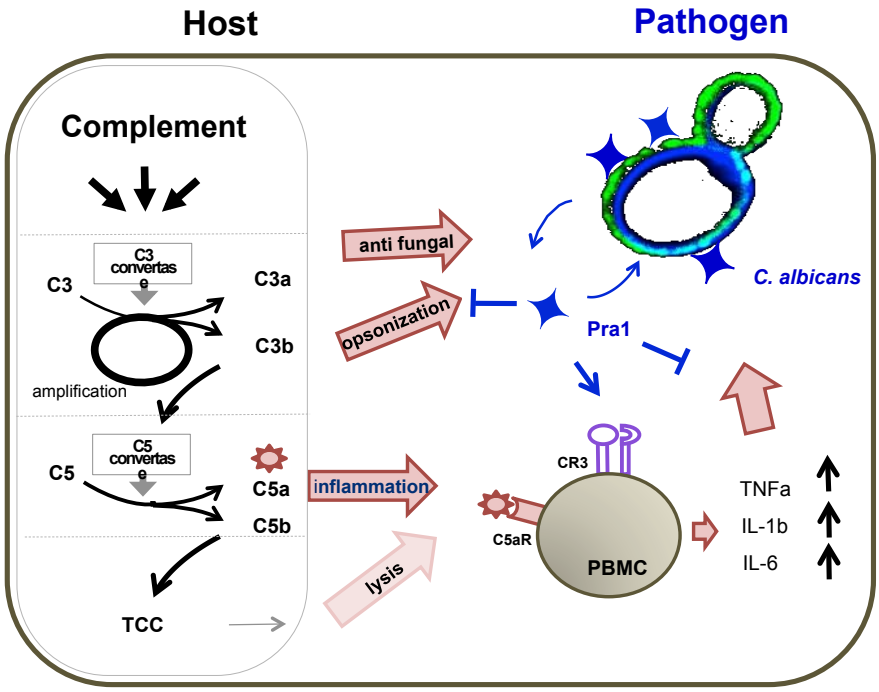

Figure 1
Host innate immunity interaction with pathogens. Complement is a central innate immune effector element of human innate immune defence. Complement is activated by three major pathways (indicated by arrows; AP, alternative pathway, LP, lectin pathway, CP, classical pathway). Each pathway forms a C3 cleaving enzyme, termed C3 convertase, which generates the activation fragments C3a and C3b. C3a has antifungal and antimicrobial activity, and C3b can deposit onto nearby surfaces. On the fungal surface - and in the absence of regulators - C3 convertase formation is amplified and this amplification loop cleaves more C3, generates additional antifungal C3a and enhances surface opsonisation with C3b. Similarly when the cascade progresses a C5 cleaving enzyme is formed, i.e. C5 convertase which generates inflammatory C5a and C5b, which initiate the terminal complement pathway.

quence block or modulate the individual effector mechanisms of the activated innate as well as adaptive host immune system. The Department of Infection Biology has isolated several innate immune evasion proteins of the fungal pathogen *Candida albicans* and we are currently characterising these as multipurpose fungal immune molecules to describe their action in detail.

One of such important multipurpose immune evasion molecules is Pra1, which was initially identified as pH regulated antigen 1. Pra1 is expressed on the fungal surface and is also secreted to the surrounding medium. Secreted Pra1 acts in fluid phase, binds back to the fungal surface, of both yeast cells and hyphae, and in addition Pra1 binds to specific receptors that are expressed at the surface of human immune effector cells (Figure 2). Pra1 binds a battery of host proteins, including complement proteins and extra cellular matrix components, and Pra1 interacts with complement receptors expressed on the surface of human macrophages and neutrophils. These human Pra1 ligands include (i) the complement regulators Factor H and FHL1, two plasma proteins that regulate the alternative complement pathway, (ii) C4BP, the soluble regulator of the classical pathway regulator, (iii) C3, a central complement protein and several C3 activation fragments, (v) CR3 (CD11b/CD18), an integrin which as a central inflammatory receptor is expressed on all myeloid cells, (vi) glycans, (vii) plasminogen, a coagulation cascade component, and (viii) fibrinogen, an extracellular matrix protein.

This interaction with multiple human immune effectors molecules, extracellular matrix components and one central inflammatory receptor shows that *Candida* Pra1 controls multiple steps of the host immune response and thus defines Candida Pra1 as a central virulence factor. Thus *Candida* Pra1 represents a fungal multipurpose protein that

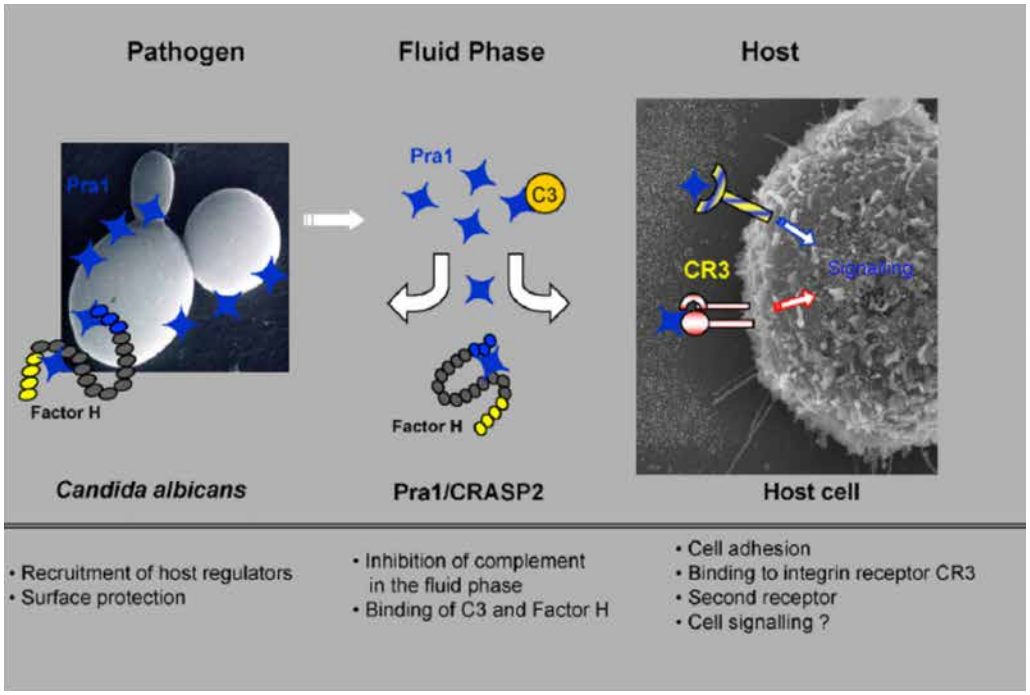

Figure 2
Candida Pra1 acts at distinct sites as a multifunctional multipurpose immune evasion tool. *Candida* pH regulated antigen is a surface protein and expressed by both yeast and hyphae. Pra1 is also secreted and the secreted Pra1 protein binds back to the surface of *Candida* and also interacts with specific receptors that are expressed on the surface of human immune effector cells. At the surface of yeasts and hyphae Pra1 binds host immune regulators, including Factor H, C4BP and plasminogen. Bound immune regulators are functionally active and aid in immune control. As a secreted protein Pra1 e.g. binds to the central complement component C3 and blocks C3 activation and thus inflammatory and cell mediated complement effector function. Pra1 also binds to the integrin and CR3 receptor on the surface of human neutrophils and directs effector function of these important immune effector cells.

controls, affects and blocks host immune response at multiple levels.

2 Immune evasion mechanisms of human pathogenic microbes

Group Leaders:
Peter F. Zipfel, Christine Skerka

Human pathogenic microbes express specific surface proteins which bind host complement and immune regulators. It is the interest of our group to identify such surface proteins, to characterise their role in immune and complement evasion and extrapolate which immune evasion strategies are common to pathogenic microbes. An emerging feature is that these pathogen surface proteins bind multiple ligands and several host proteins such as complement regulators, effector molecules of the coagulation cascade and also extracellular matrix components. These pathogen-derived complement escape proteins have a rather conserved binding profile for host immune regulators, and they bind the attached human proteins at the same site and utilise the attached host proteins for immune evasion mechanisms.

However despite this functional similarity the individual microbial evasion proteins have a high degree of sequence and antigenic diversity, between different microbes and various species, and also between individual strains.

The Gram-negative bacterium *Borrelia burgdorferi* expresses a total of five CRAS proteins (complement regulator acquiring surface proteins) and host regulator binding correlates directly with CRAS protein expression. The various *Borrelia* CRAS proteins show both unique and common binding characteristics. CRASP-1 and CRASP-2 bind Factor H and FHL-1, but not CFHR1. In contrast CRASP-3,

CRASP-4 and CRASP-5 bind Factor H and CFHR1, but not FHL-1. These binding specificities suggest a unique role for an individual CRASP protein in the immune response of *B. burgdorferi*.

A detailed binding analysis showed that the host regulator Factor H has a two point interaction and FHL-1 a one point interaction with BbCRASP1. Factor H binds to CRASP1 and CRASP2 with domain SCR7 (short consensus repeat 7) and with the C-terminal recognition domain. Consequently in its bound conformation the complement regulatory region located in the N-terminal four domains is accessible, flexible and functionally active. By expressing multiple, distinct surface proteins which have unique binding characteristics, *Borrelia* provide several interaction sites for the host regulators. Thus by increasing the number of attachment points, surface decoration with host proteins allows flexibility and displays various possible interaction sites.

Also the serum resistant forms of the pathogenic bacterium *Pseudomonas aeruginosa* bind the host immune regulators Factor H, FHL1 and CFHR1. The immune evasion strategy of this Gram-negative bacterium is different from that of *Borrelia*, as this pathogen utilises two separate strategies to control complement activation at its surface. The pathogenic *Pseudomonas* express endogenous surface proteins that inhibit complement activation and inactivate C3b, which is generated on the bacterial surface. In addition *P. aeruginosa* expresses surface proteins that act as receptors for host immune regulators and that attach to complement Factor H, FHL1 and CFHR1, and additional members of the Factor H protein family. An additional rather conserved feature of microbial pathogens is binding of the human coagulation zymogen plasminogen, which forms a central protease in the coagulation cascade when activated to plasmin.

The list of bacterial and fungal immune evasion proteins is currently increasing, and so is the identification of host ligands and the role of these microbial proteins in immune evasion. Novel bacterial binding proteins and novel interactions were recently described also for the *Streptococcus pyogenes* Scl proteins, which bind CFHR1, the human C5 convertase and TCC inhibitor. Similarly also the *Haemophilus influenzae* HIC protein binds vitronectin and plasminogen. Plasminogen bound to HIC and to the surface of *S. pyogenes* can be activated to plasmin and then controls complement by processing the central component C3. It also aids in the degradation of extracellular matrix components such as fibrinogen.

Furthermore, serum resistant Gram-positive *Streptococcus pneumoniae* express surface proteins that bind Factor H and FHL1. The two pneumococcal CRAS proteins, which are also termed PspC and HIC, were utilised to characterise the binding and interaction between the bacterial and the host proteins. Apparently both host immune effector proteins contact the pneumococcal proteins at two sides and form a two point interaction. In addition *S. pneumoniae* uses the bacterial immune evasion proteins together with the attached host immune regulators as bridging molecules to allow attachment to host cells and to bind to specific immune receptors.
This feature of acquisition of host innate immune regulators is used by a large panel of pathogenic microbes, including Gram-negative, Gram-positive bacteria as well as fungal pathogens.

In summary, evasion of complement attack is a common feature used by a large panel of pathogenic microbes. Pathogens utilise functionally related surface proteins, as they bind host plasma proteins and the complement regulators Factor H, FHL1, CFHR1 and C4BP, interact with complement components like C3 and C3 activation fragments iC3b, C3b and C3d, plasminogen, extracellular matrix components in form of fibrinogen, laminin and vitronectin, the coagulation protease plasminogen as well as with other host proteins (Figure 3). The detailed characterisation on the molecular level identified common bind-

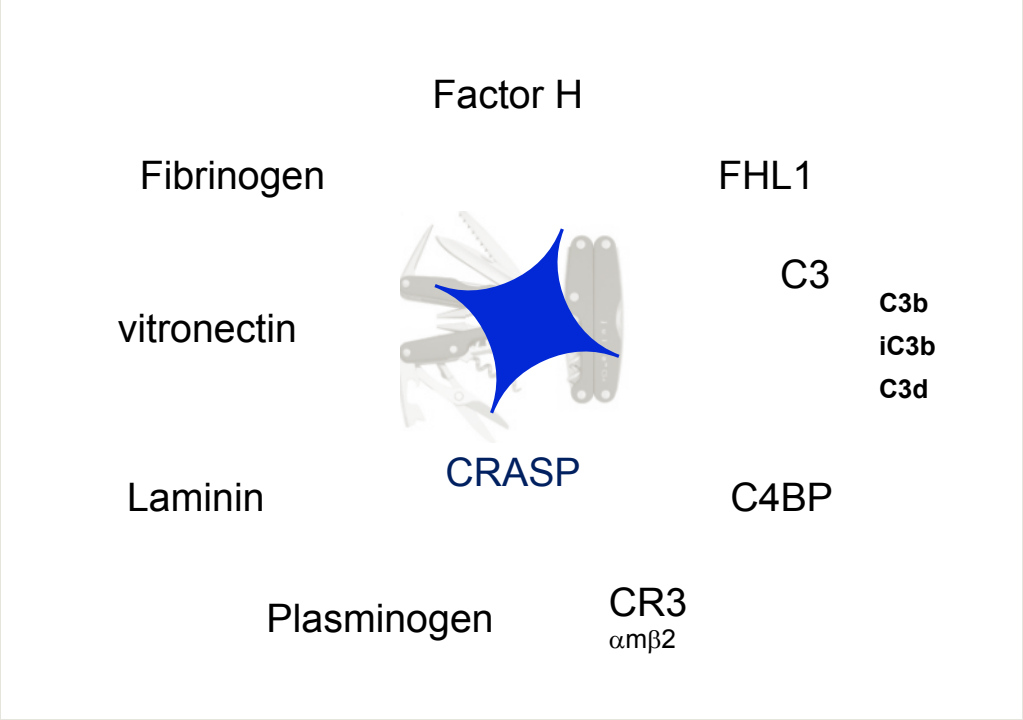

Figure 3
Pathogen derived CRAS proteins represent microbial multipurpose immune evasion tools. Pathogenic microbes express several CRAS proteins (complement regulator acquiring surface proteins) that act as multipurpose immune evasion tools. The binding profile of host regulators is rather conserved among CRAS proteins. Microbial CRAS proteins bind human complement regulators (Factor H, FHL1, CFHR1, C4Bp), coagulation proteins like plasminogen, and extra cellular matrix components (fibrinogen, laminin and vitronectin). CRAS proteins that are characterised from different pathogenic microbes include fungal pathogens (like *Candida* Pra1), Gram-positive bacteria (*S. aureus* Sbi and Efb), Gram-negative bacteria, such as *Borrelia* species (CRASP1 - CRASP5), S. *pneumoniae* (Hic and PspC) and *Haemophilus influenzae* (HIC).

ing characteristics as well as general immune evasion strategies of these microbes. Common features of this diverse group of CRAS proteins reveal that:

- (i) Pathogenic microbes utilise several different surface proteins for immune evasion,
- (ii) The individual microbial proteins bind multiple host plasma proteins,
- (iii) Individual CRSA proteins are highly polymorphic and show significant sequence variability between different strains and isolates,
- (iv) Attached host regulators are functionally active and inhibit complement, and influence interaction and attachment to host immune effector cells as well as to endothelial cells.

3 Immunoregulation

Group Leader: Christine Skerka

Beta2-Glycoprotein – the two sides of a human plasma protein

The group of Immunoregulation addresses the issue of self protection of host cells and aims to characterise novel human immune regulators and to define their role in health and in autoimmune as well as infectious diseases with *Candida albicans*.

Infections with microbes challenge the human immune system and defence mechanisms such as the complement system or the recruitment of macrophages and neutrophils are immediately activated. Toxic activation products are generated to eliminate the invading microbes and often these activation products also harm host tissues. To restrict damage to the human cells protective proteins are expressed that can differentiate be-

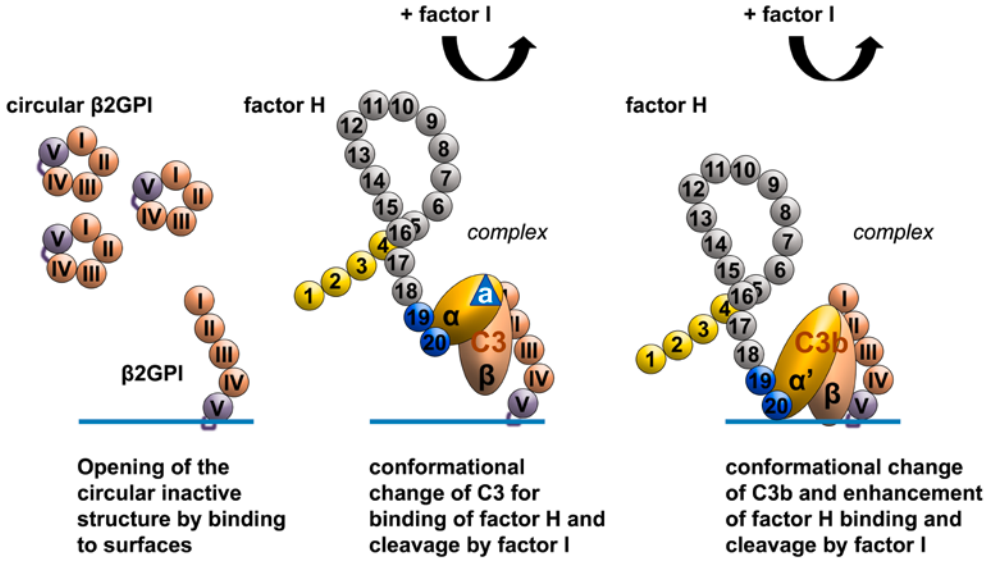

Figure 4
Plasma protein b2GPI harbours complement regulatory activities. b2GPI, the major target protein of autoantibodies in anti-phospholipid syndrome is a human plasma protein which exerts fibrinolytic activities and enhances phagocytosis. It has an inactive circular form in the plasma and opens to an elongated molecule upon surface binding. In the open form b2GPI binds C3 and C3b and mediates degradation of these complement components by Factor H and Factor I.

tween self and foreign surfaces. These proteins often recognise cell surface structures and attach to them in order to inactivate and terminate immune reactions directed to self structures. Regulatory host proteins in body fluids and also anchored proteins on the surface of human cells ensure fast inhibition of activated complement components and terminate the complement activation cascades. The importance of this regulation is reflected in several autoimmune diseases that develop on the basis of defect or missing complement regulators. Age related macular degeneration (AMD), a frequent eye disease in elderly people, as well as severe kidney diseases such as hemolytic uremic syndrome (HUS) and membranoproliferative glomerulonephritis (MPGN) are associated with impaired complement control.

One of the human plasma proteins, Beta2-Glycoprotein (b2GPI), also termed apolipoprotein H, is a human plasma protein that shows similar structural domains to the complement regulators Factor H and CFHRs. b2GPI is a relatively highly concentrated plasma protein (200 µg/ml – 4 µM) which displays anticoagulant activities. The protein inhibits the contact activation of the intrinsic coagulation pathway, platelet prothrombinase activity and ADP-induced platelet aggregation. b2GPI, like CFHR1 or CFHR3, is composed of five SCR domains and in addition harbours a binding domain to bind to human cells. Thus a function of b2GPI beyond the coagulation system was anticipated. The structural and sequence homology to Factor H family proteins suggested a role of b2GPI in complement regulation. In the autoimmune disease antiphospholipid syndrome (APS) autoantibodies to b2GPI are identified. APS is characterised by recurrent vascular thrombosis and pregnancy loss and in pregnant women b2GPI-autoantibodies trigger severe complications, resulting in miscarriage, intrauterine growth restriction and fetal death.

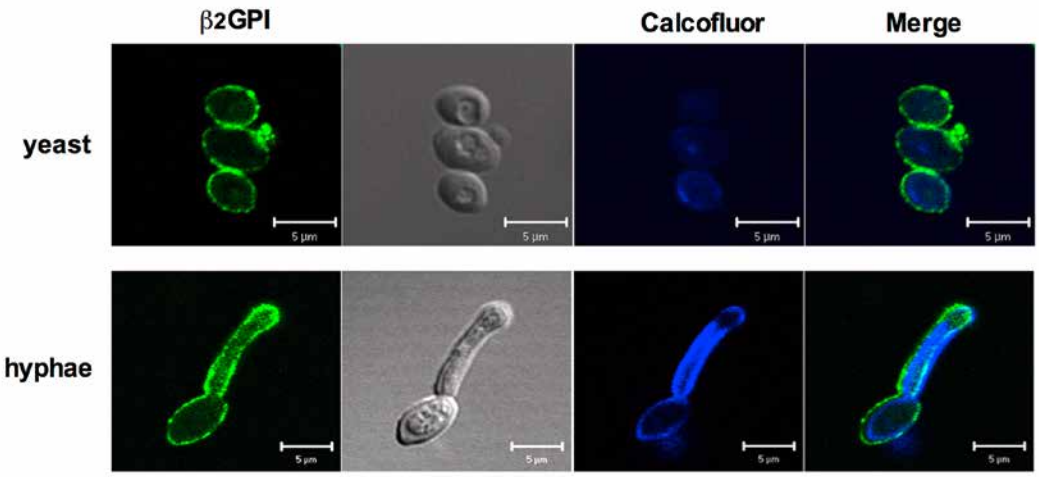

Figure 5
The human plasma protein b2GPI binds to *C. albicans* yeast and hyphae forms. b2GPI (green) binds to the surface of *C. albicans* yeast (upper panels) and to hyphae (lower panels) as show by laser scanning microscopy. *C. albicans* is stained with calcofluor (blue) and size markers (5 µm) are indicated.

To identify the role of b2GPI, the protein and protein fragments were expressed recombinantly and the function was investigated in complement assays. They revealed that b2GPI indeed exerts complement regulatory functions (Gropp *et al.*, 2011). b2GPI circulates in the plasma in an inactive circular form. Upon surface binding, such as to apoptotic cells, b2GPI changes conformation to an elongated form that binds C3/C3b. Upon binding, b2GPI apparently induces a conformational change of C3, so that the regulator Factor H attaches and allows subsequent degradation by the protease Factor I. In the presence of b2GPI C3 and also C3b are further processed to C3d. Inactivation of C3b on self surfaces is highly relevant to inhibit the generation of further convertases and to restrict depositions that may act inflammatory. On surfaces without membrane bound regulators like CR1 and in situations of high complement activity soluble regulators such as b2GPI or Factor H need to attach and clear the surfaces from complement activation products. Thus the investigations provided important insights into the functions of this plasma protein and the underlying disease mechanism of APS.

As recruitment of human complement regulators by pathogenic microbes is a widely used mechanism to evade immune reactions like complement attack, b2GPI was also tested for attachment to *C. albicans* cells. Indeed, first experiments showed that b2GPI was bound from the plasma also to the surface of *C. albicans*, as seen by laser scanning microscopy (Figure 4).

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Infectious Microbes Cause Diseases

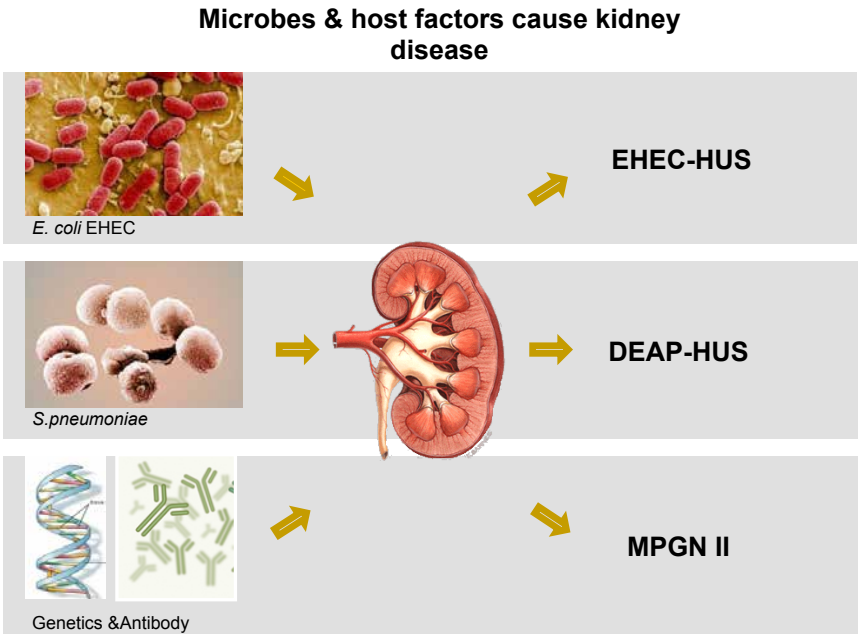

Figure 6
Infections, genetic and autoimmune forms as cause for the kidney disorders HUS and MPGN. The two human kidney disorders HUS (hemolytic uremic syndrome) and MPGN (membranoproliferative glomerulonephritis) represent different spectral outcomes of a common disease principle. HUS is caused by infections, including enterohemorrhagic *E. coli*, (EHEC_HUS) as well as *S. pneumoniae* and *Shigella dysenteria* or viruses. Similarly, genetic modifications including mutations and polymorphisms of several complement genes cause the same or highly related pathological changes. In addition autoantibodies that are formed to specific immune components result in the same pathological changes. (DEAP-HUS: Deficient for CFHR proteins and autoimmune positive form of HUS).

4 Complement dysfunction in kidney disorders

Group Leaders:
Peter F. Zipfel, Christine Skerka

Hemolytic uremic syndrome (HUS), as well as membranoproliferative glomerulonephritis (MPGN), also referred to as dense deposit disease (DDD), represent severe and rare kidney disorders that primarily affect children and young adults. Currently there is a debate whether HUS and MPGN represent different spectral outcomes of a common pathological principle. HUS and MPGN both have genetic as well as autoimmune causes. For HUS infections with *E. coli* such as enterohemorrhagic *E. coli* (EHEC) are frequent, and in the year 2011 a severe outbreak occurred in the northern states of Germany. Additional infection-associated causes of both typical and atypical HUS have been linked to infections with *Streptococcus pneumoniae* and *Shigella dysenteria*. For about 60 – 70 % of patients genetic causes have been identified and most frequently include heterozygous mutations in the genes coding for the C3 convertase regulators, Factor H, Factor I, Membrane Cofactor Protein (MCP/CD46), thrombomodulin, as well as the C3 convertase components C3 and Factor B. In addition, an autoimmune form exist which frequently manifests in patients at the age of 5 to 17 years. Frequently the autoantibodies bind to the C-terminus of Factor H and block Factor H surface attachment. In several cases the autoantibodies develop on a genetic constellation in form of the homozygous deletion of a chromosomal 84 kb segment in the CFHR gene cluster on human chromosome 1. Patients with the different kidney disease forms (infection associated, genetic or autoimmune) are identified by specific diagnosis and the various disease forms often only respond to specific therapy. Treatment options include anti-infective therapy, complement inhibition, plasma exchange, as

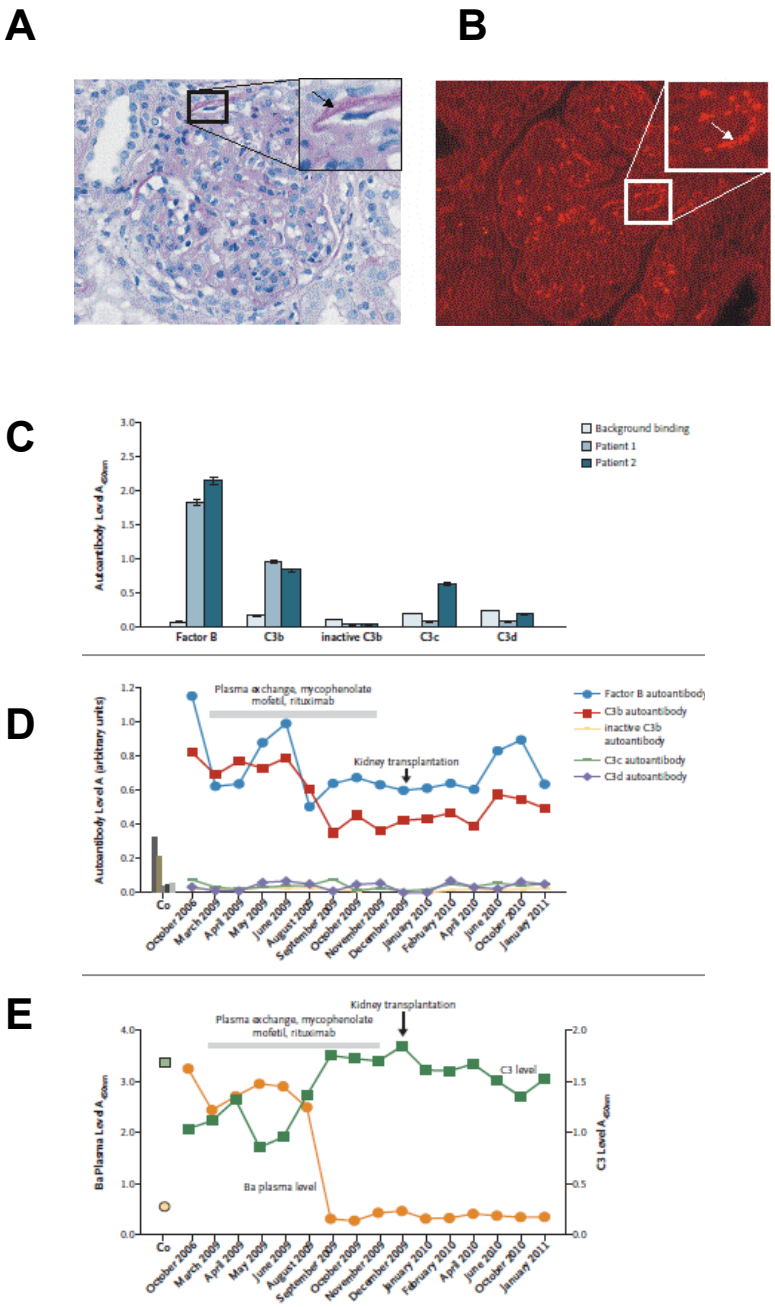

Figure 7
Pathology, characterisation of Factor B- and C3 autoantibodies and treatment in autoimmune MPGN II.
(A) PAS staining of renal biopsy of the eight-year-old patient shows a typical pattern of MPGN II, i.e., thickening of the basal membrane (arrow, inset) and mesangial cell proliferation.
(B) Immune-staining revealed deposition of complement C3 to the basal membrane (arrow, insert).
(C) Binding profile of autoantibodies of the eight-year-old patient (light purple columns) and the twenty-year-old patient (dark purple columns) to Factor B (FB), C3b and C3 activation fragments. Background binding is indicated (open columns).
(D) Factor B (blue circles) and C3b (red squares) autoantibody levels in plasma of the 8-year-old patient declined upon immunosuppressive therapy and stayed low after kidney transplantation.
(E) shows the changes of C3 (green squares) and Ba plasma levels (orange circles) of the 8-year-old patient prior, during and after immunosuppressive therapy and following kidney transplantation. C3 and Ba levels in NHS are indicated (Co).

well as reduction of autoantibodies by immunosuppressive therapy (Figure 6).

Similarly membranoproliferative glomerulonephritis type II (MPGN II) is a complement-associated severe kidney disease. It is due to systemic C3 activation and deposition of C3 cleavage products along the glomerular basement membrane and often progresses to end-stage renal disease (ESRD). Genetic causes include mutations in the genes encoding for complement Factor H and C3, which result in deregulation of the C3 convertase. In addition, autoantibodies, such as C3 Nephritic Factor (C3Nef), which stabilise C3 convertase and induce permanent complement activation, have been identified in 50 – 80 % of MPGN II patients. Patients with autoantibodies to Factor H have also been described. There is currently no effective treatment for MPGN II patients, and the prognosis for survival of a kidney transplant is poor.

We have identified two unrelated patients – an eight-year-old girl with MPGN II and ESRD and a twenty-year-old man with MPGN – with novel autoantibodies, in the form of combined Factor B and C3 autoantibodies

(Figure 7C). Both patients lacked C3Nef, but presented autoantibodies to the two individual components of the C3 convertase, i.e. C3b and Factor B. These autoantibodies enhanced C3 convertase activity, as demonstrated by higher levels of cleavage products Ba and C3a upon addition of isolated IgGs from both patients to normal human serum (NHS) or to the *in vitro* assembled C3 convertase. The eight-year-old girl showed heterozygous *CFHR1* and *CFHR3* deficiency. Thus these novel autoimmune MPGN patients have autoantibody development independent of homozygous complement Factor H-related protein gene deletion in contrast to patients with atypical hemolytic uremic syndrome (DEAP_HUS).

Based on the diagnosis of autoimmune MPGN II the eight-year-old patient received weekly plasma exchanges and immunosuppressive therapy and consequently her C3b autoantibody levels declined and her Factor B autoantibody levels decreased (Figure 7D). In addition the Ba activation fragment dropped to background levels, and C3 plasma levels increased to normal values (Figure 7E). Following the reduction of the autoantibodies titers, this patient received a kidney transplant in December 2009 and since that time serum creatinine and proteinuria are absent and the patient is stable and without disease recurrence. This novel autoimmune form of MPGN shows that in addition to C3Nef further autoantibodies develop in MPGN II and lead to complement deregulation that requires targeted treatment.

(On behalf of the European Committee on MPGN)

5 Complement dysfunction in age related macular degeneration (AMD)

Group Leaders: Christine Skerka,
Peter F. Zipfel

Age related macular degeneration (AMD) is the leading cause of blindness in the elderly population in Western societies. Approximately 20 million individuals in Europe and in the United States suffer from this sight-threatening disease. Late disease stages present in two severe forms that both result in central vision loss. Geographic atrophy is caused by atrophy of photoreceptors in the macular area that overlie degenerated retinal pigment epithelial (RPE) cells. Choroidal neovascularisation develops due to the growth of new blood vessels into the retinal layer. A hallmark of AMD and usually one of the first clinical symptoms is the presence of ocular drusen. Proteomic and histochemical analyses show that these extracellular deposits contain complement components and inflammatory proteins.

During the last years, mutations in several complement genes have been linked to AMD. Genetic polymorphisms in genes coding for Factor H, Factor B, C2, C3 as well as a 84 kbp chromosomal deletion that results in the absence of the complement regulators CFHR1 and CFHR3 significantly influence AMD pathogenesis. One prominent AMD-associated polymorphism within the Factor H gene is a T $\rightarrow$ C substitution at nucleotide 1277 which results in a tyrosine (Y) to histidine (H) exchange at amino acid position 402. The Factor H risk variant H402 increases the risk for AMD about 2-4 fold for heterozygote and 5-7 fold for homozygote individuals.

The human Factor H gene encodes two proteins: Factor H itself and the Factor H-like protein (FHL1). Both plasma proteins are synthesised in the liver but are also expressed locally, e.g. in RPE cells. Factor H is composed of 20 consecutive protein domains, termed short consensus repeats (SCRs). FHL1, which is derived from an alternatively spliced transcript, represents the seven N-terminal SCRs of Factor H and has a unique C-terminal extension of four amino acids. Both Factor H and FHL1 include the AMD relevant residue 402 in SCR 7 and are major regulators of the alternative complement pathway. The two proteins act as cofactors for Factor I mediated C3b inactivation and accelerate the decay of

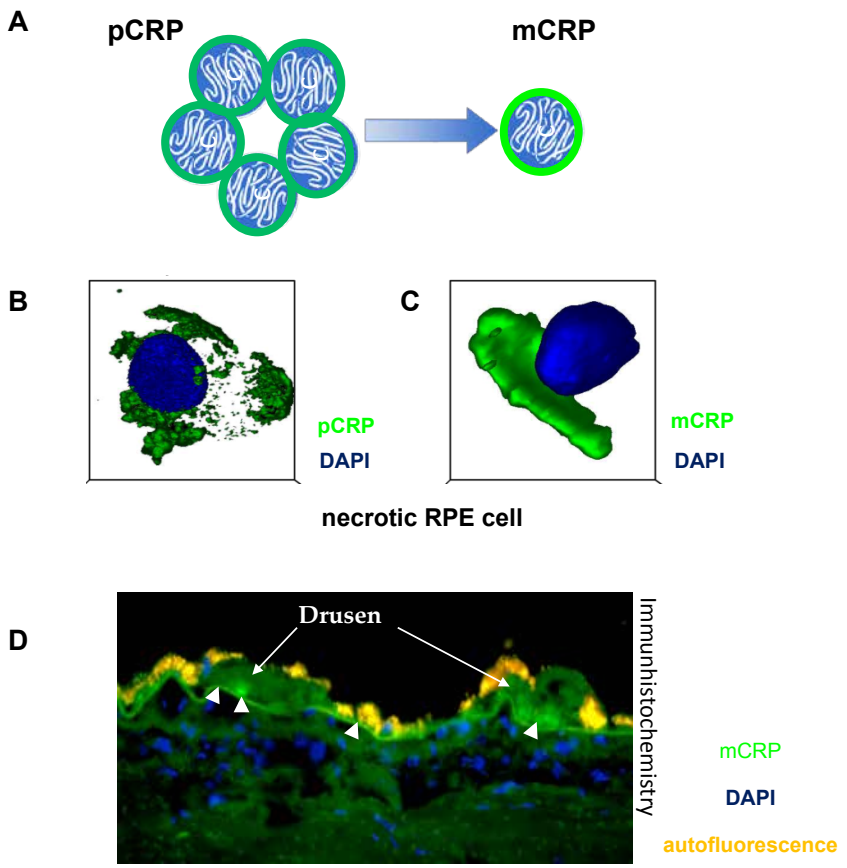

Figure 8
The inflammatory marker mCRP is formed at sites of cellular damage. **(A)** The inflammatory marker CRP circulates as a pentamer (pCRP), and when attached to a surface is dissociated into the monomeric variants (mCRP). (B - C) Different localisation and cell surface distribution of pCRP **(B)** and mCRP **(C)** at the surface of necrotic retinal pigment epithelial cells. **(D)** mCRP is present in retinal-choroidal tissues of AMD patients. Tissues derived from a patient with clinically documented AMD was fixed and incubated in a monoclonal mouse antibody to mCRP (CRP-8). Bound antibodies were visualised using an Alexa488-conjugated secondary antiserum (green). Nuclear counterstaining was performed with propidium iodide (red).

a preformed C3bBb convertase. Both regulators possess multiple binding sites for C3b, Heparin, C-reactive protein (CRP) and bind to cellular surfaces. In this context, SCR 7 binds to the ligands heparin and CRP and mediates cell surface binding. The non-risk associated variant Y402 of Factor H and of FHL1 binds stronger to CRP as the H402 risk variant, suggesting a role of this AMD-associated polymorphism for ocular inflammation.

Individuals who are homozygous for the Factor H H402 risk variant show 2.5-fold higher CRP levels in the RPE-choroid layer, as compared to individuals homozygous for the non-risk associated Y402 variant.

CRP is an acute phase protein which is distributed in circulation in the 125 kDa pentameric form (pCRP) and the five identical subunits are stabilised by calcium ions (Figure 8A). pCRP is modified upon inflammation, surface attachment, oxidative stress, low pH, proteolytic cleavage, calcium depletion and *in vitro* by heat or by urea treatment. Ultimately pCRP dissociates into the monomeric units of 23 kDa, termed mCRP. mCRP is deposited at sites of immune stress; and at the surface of damaged, i.e. necrotic retinal pigment epithelial cells, mCRP is localised at specific and unique sites. Surface bound mCRP serves as a platform to bind complement regulators such as Factor H, FHL1 and C4BP and other anti inflammatory compounds (Figure 8B and C).

Thus specifically mCRP, but not pCRP, recruits a battery of complement soluble regulators to the surface of apoptotic cells and apoptotic particles. These attached regulators control complement progression both of the alternative and the classical pathway to allow formation and to moderate action of the C3 convertase, and in contrast to inhibit excessive activation and amplification of the complement cascade. This results in the

deposition of C3b and opsonization to allow enhanced and efficient phagocytosis and clearance. In addition the attached regulators have an anti-inflammatory activity, and reduce the release of the pro-inflammatory cytokine TNF-α and IL-8.

mCRP is identified in drusen and ocular tissues and this presence is indicative of chronic local inflammation within retinal layers. Persistent local inflammation near ocular drusen damages RPE cells and causes cell swelling, pigmentation and changes in organelle distribution. Ultrastructural and histochemical analyses show that in AMD patients, RPE cells in the neighborhood of drusen degenerate due to necrosis. Rupture of the plasma membrane is a characteristic feature for necrotic cell death and is followed by release of cytoplasmatic contents in form of DNA, histones and other proteins which initiate and further amplify local inflammation within the retina and attract blood-derived macrophages (Figure 8D).

Within retinal tissues derived from AMD patients both pCRP and mCRP are located at the Buchs membrane and in the epithelial layer of pigment cells. Thus mCRP is generated *in vivo* and this newly generated protein is localised at distinct patches on necrotic cells and co-localises with the cell damage marker annexin V. The non-risk associated Y402 variant of Factor H forms strong complexes with mCRP and is recruited by mCRP to such necrotic lesions. Recruited Factor H maintains complement regulatory activity, efficiently inactivates complement and blocks release of pro-inflammatory cytokine TNF-α. Reduced mCRP binding of the Factor H H402 risk variant results in complement activation, generation of inflammatory mediators, inflammation and finally in pathology. In conclusion, mCRP has a physiological, anti-inflammatory function on retinal cells and the reduced interaction between mCRP and the risk variants of Factor H and FHL1 (i.e. Factor H_{H402} and $FHL1_{H402}$) has functional effects that explain AMD pathology and can be exploited for therapy.

The Factor H related proteins (CFHR1 – CFHR5) are plasma proteins whose role in complement activation and regulation is poorly understood. Five members of this complement Factor H family are encoded by separate genes, which are located in the Factor H gene cluster, adjacent to the Factor H gene *CFH*. The common feature of this family of secreted proteins is their exclusive composition of individual domains called short consensus repeats (SCR). Different degrees of amino acid identity to each other and to Factor H among the various CFHR proteins indicate complement regulatory functions of the CFHR proteins.

Interestingly the homozygous deletion of a 84 kbp chromosomal fragment downstream of *CFH* that includes the genes CFHR1 and CFHR3 represents a risk factor for systemic lupus erythematosus (SLE). However the same deletion is protective for AMD. In addition a polymorphism within the gene coding for Factor H (rs2274700) is associated with a substantially higher risk to develop AMD. As all three genes are located in the gene cluster of complement regulators on chromosome 1 linkage disequilibrium of the deletion with the protective variant of Factor H was suggested. To investigate whether CFHR1 and CFHR3 are independent factors that influence the risk of AMD, a collaborative project with Professor Weber from the Institute of Human Genetics of the University of Regensburg was performed. This work demonstrated that in a huge German cohort of AMD patients the protective effect by the deletion of the CFHR1 and CFHR3 genes is independent of the polymorphism (rs2274700) in the Factor H gene. Functional studies with the CFHR1 and CFHR3 proteins underlined the genetic results, as both proteins were able to compete with Factor H in binding and functional activities. In conclusion the studies demonstrate a tight balance between the complement regulators CFHR1, CFHR3 and Factor H and show that imbalances due to genetically caused deficiencies affect the risk for the autoimmune disease AMD.

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Jena School for Microbial Communication
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Role of Complement CRP complexes in Inflammation and Organ Dysfunction
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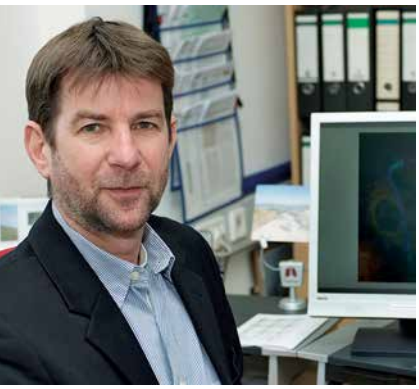

The Department of Microbial Pathogenicity Mechanisms (MPM) is investigating infections caused by human pathogenic yeasts, in particular *Candida albicans* and *Candida glabrata.*

Our research is supported by the Jena School for Microbial Communication (JSMC), the International Leibniz Research School (ILRS) for Microbial and Biomolecular Interactions, the Deutsche Forschungsgemeinschaft (DFG), the Studienstiftung des Deutschen Volkes, the Federal Ministry of Education and Research (BMBF), the Framework 7 programme of the European Union and in parts by companies such as Bayer Vital GmbH or Biolitec AG. Projects are integrated into the Center for Sepsis Control and Care (CSCC), the DFG Priority Program SPP1580 "Intracellular compartments as places of pathogen-host-interactions", the project "Microevolution of pathogenic yeasts during interactions with the host" within the DFG DACH programme, and international networks such as the European CandiCol consortium and the European FINSysB network.

Candida infections

In contrast to most pathogenic fungi of humans, such as *Aspergillus fumigatus*, *Cryptococcus neoformans* and *Histoplasma capsulatum*, which are found in the environment, *Candida albicans* and *C. glabrata* belong to the normal microbial flora of the skin, intestinal tract or other mucosal surfaces, and are regarded as harmless commensals under most circumstances. In fact, these commen-

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Head:
Prof. Dr. Bernhard Hube

Die Abteilung Mikrobielle Pathogenitätsmechanismen (MPM) erforscht die Ursache und Entwicklung von Infektionen durch human-pathogene Hefen, insbesondere *Candida albicans* und *C. 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 die Jena School for Microbial Communication (JSMC), die International Leibniz Research School (ILRS), die Deutsche Forschungsgemeinschaft (DFG), die Studienstiftung des Deutschen Volkes, durch das Bundesministerium für Bildung und Forschung (BMBF) und das 7. Rahmenprogramm der Europäischen Union. Die Projekte sind integriert in das Center for Sepsis Control and Care (CSCC), das DFG-Schwerpunktprogramm SPP1580 "Intracellular compartments as places of pathogen-host-interactions", das Projekt "Microevolution of pathogenic yeasts during interactions with the host" innerhalb des DFG DACH-Programms und in Netzwerke wie das Europäische CandiCol-Konsortium und das Europäische FINSysB-Netzwerk.

Candida-Infektionen

Im Gegensatz zu vielen anderen krankheitserregenden Pilzen des Menschen, wie zum Beispiel *Aspergillus fumigatus*, *Cryptococcus neoformans* und *Histoplasma capsulatum*, welche normalerweise nur in der Umwelt zu finden sind, zählen *Candida albicans* und

sal yeasts can be detected in up to 71 % of the healthy population.

An intact immune system, a balanced microbial flora and the epithelial barriers are normally sufficient to protect an individual from *Candida* infections. However, certain critical events such as 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. *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. catheters). From the bloodstream, cells can infect every organ of the body (invasive candidosis), but appear to prefer certain organs depending upon the route of infection. 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.

Candida albicans

The yeast *Candida albicans* is regarded as

C. glabrata zur normalen mikrobiellen Flora der Schleimhäute und gelten in der Regel als harmlose Kommensale. Tatsächlich können diese kommensalen Hefen bei bis zu 71 % der gesunden Bevölkerung gefunden werden.

Ein intaktes Immunsystem und eine gesunde mikrobielle Flora sind normalerweise ausreichend, um einen Menschen vor einer oberflächlichen *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.

Candida-Zellen können durch direkte Penetration der Epithelschichten, nach Zerstörung physikalischer Barrieren – etwa aufgrund chirurgischer Eingriffe, Polytraumata oder medikamentöser Behandlungen - oder ausgehend von Biofilmen auf implantierten medizinischen Hilfsmitteln (z. B. Kathetern) das Blutgefäß-System erreichen. Ausgehend von den Blutgefäßen können die Pilzzellen praktisch jedes Organ des Menschen infizieren (invasive Candidosen). Bereits relativ geringe Immunsuppressionen reichen aus, um einen Menschen für eine invasive Candidose empfänglich zu machen. *Candida*-Pilze gelten dabei als die bei weitem häufigsten Erreger von nosokomialen (im Krankenhaus erworbenen) Pilzinfektionen.

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 human microbial flora. Superficial infections with *C. albicans* are extremely common in even mildly immunocompromised individuals. Furthermore, in hospital settings, *C. albicans* can cause life-threatening systemic infections. These infections 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, 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. 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 gene during experimental infection.

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Candida albicans

Candida albicans gilt als der medizinisch wichtigste Pilz und ist ein extrem erfolgreicher Krankheitserreger 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 *C. albicans* lebensbedrohliche systemische Infektionen hervorrufen. Während dieser Infektionen 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 Microarray-Chips, sowie durch die Entwicklung von Techniken, die relativ einfache und schnelle molekularbiologische Manipulationen des *C. albicans*-Genoms ermöglichen, wurde in den letzten Jahren eine Fülle von Informationen über die faszinierende Biologie und die Virulenzmechanismen dieses Pathogens generiert. Darüber hinaus wurde eine Reihe von Infektionsmodellen etabliert, um genomweite Genexpressionen oder die Beteiligung von ausgesuchten Genen bei Infektionen untersuchen zu können.

Candida glabrata

Das *C. glabrata* gilt als ein “emerging pathogen”, der immer häufiger in Kliniken isoliert

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 more 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*. Many genomic and molecular biology tools such as promoters, markers, reporters and even plasmids are interchangeable between the two yeasts. The known genome sequence of *C. glabrata*, mutant libraries and microarray techniques provide a solid basis for advanced molecular analysis of pathogenicity traits and mechanisms.

Our goal: elucidation 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 Microbial Pathogenicity

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 Pathogenitätsmechanismen 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. 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*, Mutantenbibliotheken und Microarray-Chip-Techniken bilden eine solide Basis für die molekulare Analyse der Pathogenitätsmechanismen dieses Pilzes.

Unser Ziel: Aufklärung der Pathogenitätsmechanismen von *Candida*-Hefen

Ziel der Forschungen der Abteilung Mikrobielle Pathogenitätsmechansimen ist es, am Beispiel von *Candida*-Hefen aufzuklären, welche Faktoren krankheitserregende Pilze benötigen, um Infektionen zu verursachen, und welche Mechanismen bei den Interaktionen dieser Hefen 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

Mechanisms is to identify factors which fungal pathogens need in order to cause disease. In addition to these efforts to increase our understanding of the basics of pathogenesis of fungal infections, the department also seeks to identify infection- and stage-specific biomarkers and new potential targets for antimycotic drug development.

Non-*Candida* research

In addition to research on *Candida*, we also study emerging fungal pathogens such as *Aspergillus terreus* or Zygomycetes. Pathogenicity mechanisms of the most common airborne fungus, *A. fumigatus*, are studied in collaboration with the department Molecular and Applied Microbiology at the HKI.

Infection models for fungal infections:

Integrated into the Department of Microbial Pathogenicity Mechanisms is a research group which carries out experimental fungal infections using a broad range of *in vitro*, *ex vivo* and *in vivo* infection models. These include simple models based on epithelial monolayers (Figure 1), epithelial tissue, whole blood, primary phagocytes and phagocyte cell lines, *ex vivo* perfused organs, alternative complex infection models and mice models.

Alternative models include the embryonated hen's egg model of *Candida* and *Aspergillus* infection, which has been developed in our department. Once mutant strains of *Aspergillus fumigatus*, *Candida albicans*, *C. glabrata* or

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verstanden, sondern auch stadienspezifische Biomarker und Ansatzpunkte für neue Medikamente gefunden werden.

Forschung an Nicht-*Candida* Arten

Zusätzlich zu Forschungen an *Candida* beschäftigen sich einige wenige Projekte mit aufkommenden Pilzpathogenen wie *Aspergillus terreus* oder Zygomyceten. Pathogenitätsmechanismen des am häufigsten über die Luft verbreiteten pathogenen Pilzes *Aspergillus fumigatus* werden in Zusammenarbeit mit der Abteilung Molekulare und Angewandte Mikrobiologie am HKI untersucht.

Infektionsmodelle zur Untersuchung pathogener Pilze

Eingebettet in die Abteilung Molekulare Pathogenitätsmechanismen ist die Forschungsgruppe „Infektionsmodelle". Forschungsschwerpunkte dieser Gruppe liegen auf der experimentellen Infektionsbiologie von Pilzinfektionen. Dazu wurde eine breite Palette von *in vitro*-, *ex vivo*- und *in vivo*-Infektionsmodellen entwickelt. Diese Modelle basieren auf einfachen Epithelzellmodellen (Figure 1), Blutmodellen, primären Phagocyten, Phagocytenzelllinien, *ex vivo* perfundierten Organen, alternativen komplexen Infektionsmodellen und Mausmodellen.

Alternative Modelle schließen das Modell des embryonierten Hühnereis ein, das in unserer Abteilung entwickelt wurde. Erst nachdem Mutantenstämme von *Aspergillus fumigatus*, *Candida albicans*, *C. glabrata* oder *A. terreus* ausreichend *in vitro* und in alternativen Modellen getestet wurden, werden Mausmodelle eingesetzt, um die Pathogenese von Pilzinfektionen in einem Säugetier untersuchen zu können. Dabei beurteilen wir die Virulenz von Pilzmutanten nicht nur anhand der

A. terreus have been sufficiently tested *in vitro* and in alternative models, mice infection models are routinely used to study virulence. Readout parameters include not only survival, but also multiple in-depth post-mortem analyses including histopathology (in collaboration with the Leibniz Institute for Age Research - Fritz Lipmann Institute), tissue burden, organ damage via blood levels of marker enzymes and determination of the cytokine response.

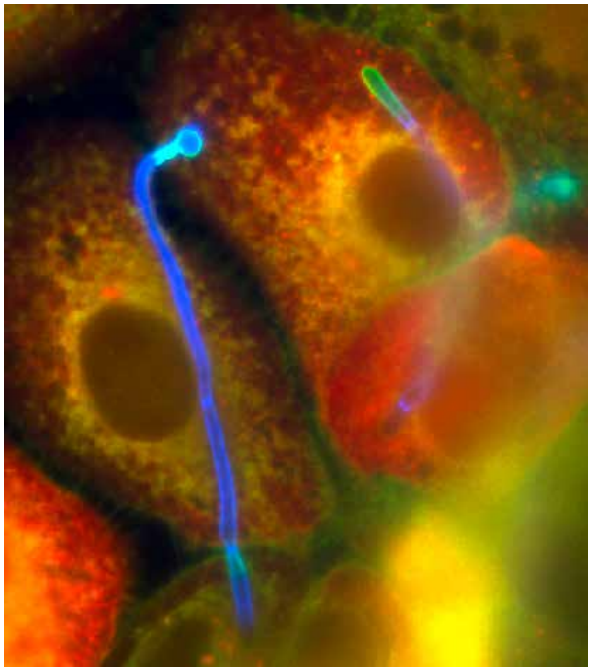

Figure 1
Interepithelial dissemination of *Candida albicans*. A mother yeast cell (bright blue) has germinated, penetrated through an human epithelial cell (dark blue), has reached the extracellular space (bright blue) and invaded the next epithelial cell (dark blue again).

Mortalität infizierter Versuchstiere, sondern nutzen zahlreiche weitere Analysen, wie z. B. Histopathologie (Zusammenarbeit mit dem Leibniz-Institut für Altersforschung – Fritz-Lipmann-Institut), Keimzahlmessungen, Messungen von Gewebsschädigungen über Markerenzyme im Blut und Bestimmung der Zytokinantwort.

1 *Candida albicans* interactions with human epithelial cells

Group leaders: Betty Wächtler, Duncan Wilson, Bernhard Hube

The human pathogenic fungus *Candida albicans* frequently causes superficial infections by invading and damaging epithelial cells, but may also cause systemic infections by penetrating epithelial barriers.

Therefore, one of the projects in our department is to investigate the interactions of *C. albicans* with human epithelial cells. In 2010 to 2011, Betty Wächtler, Ronny Martin, Duncan Wilson, Nadja Jablonowski, Stefanie Förster, Jenny Domey, Katja Haedicke, Melanie Polke, Antje Heyken, Ilse Jacobsen, and Bernhard Hube were involved in this project.

Cell type-specific interactions

One of the main reservoirs of *C. albicans* is the gastrointestinal tract and systemic infections predominantly originate from this niche. In one of our studies in cooperation with Dr Frederic Dalle, University of Dijon (France), Dr Norbert Banner and Gudrun Holland, Robert Koch Institute (Berlin, Germany), we used scanning electron and fluorescence microscopy, adhesion, invasion and damage assays, fungal mutants and a set of fungal and host cell inhibitors to investigate the interactions of *C. albicans* with oral epithelial cells and enterocytes (Dalle *et al.*, 2010). Our data demonstrate that adhesion, invasion and damage by *C. albicans* depend not only on fungal morphology and activity, but also on the epithelial cell type and the differentiation stage of the epithelial cells, indicating that epithelial cells differ in their susceptibility to the fungus. *C. albicans* can invade epithelial cells by induced endocytosis and/or active penetration. However, depending on the host cell faced by the fungus, these routes are exploited to a different extent. While invasion into oral cells occurs via both routes, invasion into intestinal cells occurs only via active penetration.

Stage-specific factors

Induced endocytosis is analogous to invasion by facultative intracellular enteropathogenic bacteria, and active penetration similar to plant pathogenic fungi.

In another study (Wächtler *et al.*, 2011a), we systematically studied the molecular basis of *C. albicans* adhesion to, invasion into and damage of oral epithelial cells. By assessing the contributions of defined fungal pathways and factors to these different stages of epithelial interactions, we provided an expansive portrait of the processes and activities involved in epithelial infection. We strengthened the concept that hyphal formation is critical for epithelial invasion. Importantly, our data support a model whereby initial epithelial invasion *per se* does not elicit host damage, but that *C. albicans* relies on a combination of contact-sensing, directed hyphal extension, active penetration and the expression of novel pathogenicity factors for further inter-epithelial invasion, dissemination and ultimate damage of host cells. Furthermore, we explored the transcriptional landscape of *C. albicans* during the early stages of epithelial interaction, and, via genetic analysis, identified, for example, Icl1 and Pga34 as novel oral epithelial pathogenicity factors.

In a previous study (Zakikhany *et al.*, 2007) we used genome-wide microarrays, *in vitro* infection models and samples from patients with pseudomembranous candidiasis and identified several genes which encode known and unknown fungal factors associated with oral infection. The expression of selected genes has been investigated via qRT-PCR in both *in vitro* models and *in vivo* samples from

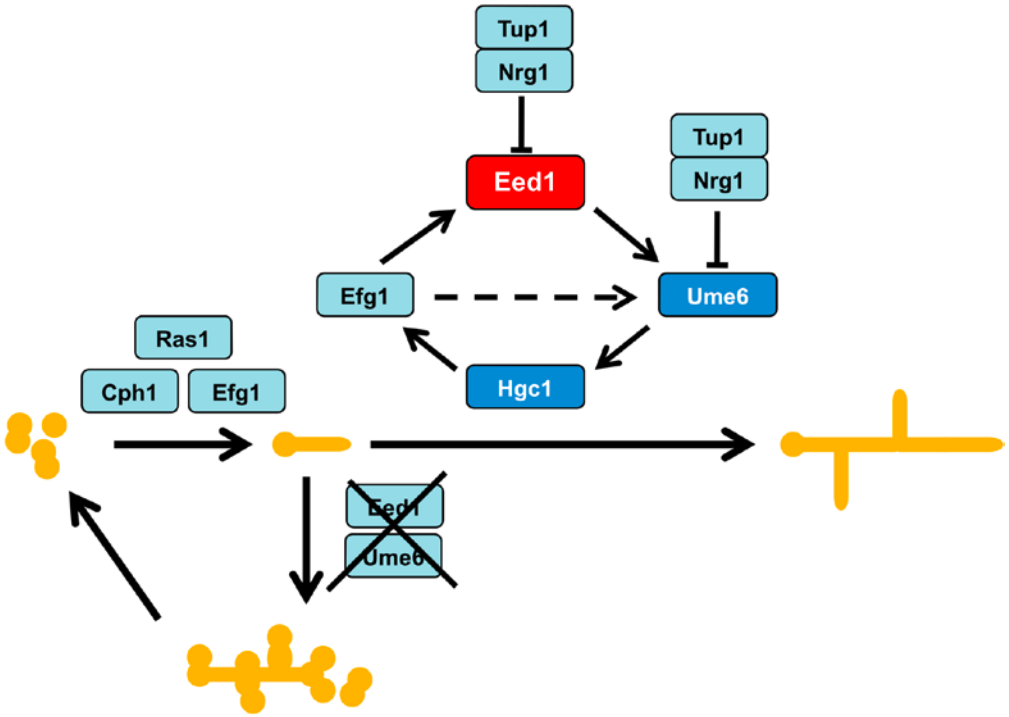

Figure 2
Regulation of hyphal extension by the key regulator Eed1. The proposed model indicates the crucial role of Eed1 during hyphal growth of *C. albicans*. Yeast cells are induced by environmental stimuli to form germ tubes. Regulators like Ras1, Cph1 and Efg1 control this process. Eed1 and Ume6 are essential for extension of initial filaments into long hyphae. The G1 cyclin Hgc1 is regulated by Ume6. Deletion of either EED1 or UME6 causes budding of yeast cells from the initial filaments and initiation of hyphal-to-yeast transition (from Martin *et al.*, 2011a).

patients. Several lines of evidence suggested that fungal morphology and morphology associated attributes play key roles in adhesion to and invasion into oral epithelial cells, and mutants lacking regulators of hyphal formation are attenuated in their ability to invade and damage epithelial cells. Adhesion is mediated by hyphal-associated factors such as Hwp1 and the Als adhesin family. Hyphal formation facilitates epithelial invasion via two routes: active penetration and induced endocytosis. While induced endocytosis is predominantly mediated by the adhesin and invasin Als3, active penetration seems to be supported by hydrolase activity and mechanical pressure. Expression profiles reflect the morphological switch and an adaptive response to neutral pH, non-glucose carbon sources, and nitrosative stress (Martin *et al.*, 2011b).

Regulation of hyphal extension

Since it was clear that the extension of germ tubes into elongated hyphae by *C. albicans* is essential for damage of host cells, we focused on the regulation of hyphal extension in another project, in collaboration with Dr Gary Moran and Prof Derek Sullivan, Trinity College Dublin (Ireland), and Prof Oliver Kurzai, Fungal Septomics (Jena, Germany), (Martin *et al.*, 2011a).

The *C. albicans*-specific gene *EED1* plays a crucial role in this extension and maintenance of filamentous growth (Figure 2). *eed1*Δ cells failed to extend germ tubes into long filaments and switched back to yeast growth after 3h of incubation during growth on plastic surfaces. Expression of *EED1* is regulated by the transcription factor Efg1 and ectopic overexpression of *EED1* restored filamentation in *efg1*Δ. Transcriptional profiling of *eed1*Δ during infection of oral tissue revealed down-regulation of hyphal associated genes including *UME6*, encoding another key transcriptional fac-

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tor. Ectopic overexpression of *EED1* or *UME6* rescued filamentation and damage potential in *eed1*Δ. Transcriptional profiling during overexpression of *UME6* identified subsets of genes regulated by Eed1 or Ume6. These data suggest that Eed1 and Ume6 act in a pathway regulating maintenance of hyphal growth thereby repressing hyphal-to-yeast transition and permitting dissemination of *C. albicans* within epithelial tissues.

Dimorphism as a therapeutic target?

Since the ability to switch between yeast and hyphal growth forms (dimorphism) is one of the most discussed and best investigated virulence attributes of *C. albicans*, we further focused our research on this topic and questioned whether dimorphism may be a therapeutic target (Jacobsen *et al.*, 2012). Both morphological forms seem to be important for virulence and have distinct functions during the different stages of disease development, including adhesion, invasion, damage, dissemination, immune evasion and host response.

Clotrimazole and bifonazole are highly effective antifungal agents against mucosal *C. albicans* infections. In one study (Wächtler *et al.*, 2011b), we examined the effects of low levels of clotrimazole and bifonazole on the ability of *C. albicans* to adhere, invade, and damage vaginal epithelial cells. Although adhesion and invasion were not affected, damage was greatly reduced upon azole treatment. This clearly indicates that low levels of azoles influence specific activities of *C. albicans* during distinct stages of vaginal epithelium infections.

Finally, we contributed to projects on *C. albicans* interactions with epithelial cells and mucosal immunity in cooperation with Dr Julian Naglik, King's College (London, UK), (Naglik *et al.*, 2011). Here, we investigated the commensal and pathogenic *C. albicans*-epithelial interactions and discussed how this may lead to the induction of a protective mucosal immune response.

This work is financed in part by the Deutsche Forschungsgemeinschaft (Hu528/10-1, 2 and 3 within the DFG Priority Programme SPP 1160 "Colonisation and Infection by human-pathogenic Fungi"), and Bayer Vital.

2 Infection-associated genes of *Candida albicans*

Group leaders: François Mayer, Francesco Citiulo, 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 dissected which genes, regulons and biological processes are relevant during different phases of *C. albicans* infection (Wilson *et al.*, 2009).

Although capable of providing 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 up-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 (Fradin *et al.*, 2005; Thewes *et al.*, 2007; Zakikhany *et al.*, 2007). We have therefore selected 50 previously uncharacterised "infection-associated genes", and analysed their function during infection (Figure 3 gives an overview of the selection and characterisation approach).

Unlike certain bacterial pathogens, where production of a single toxin is often suffi-

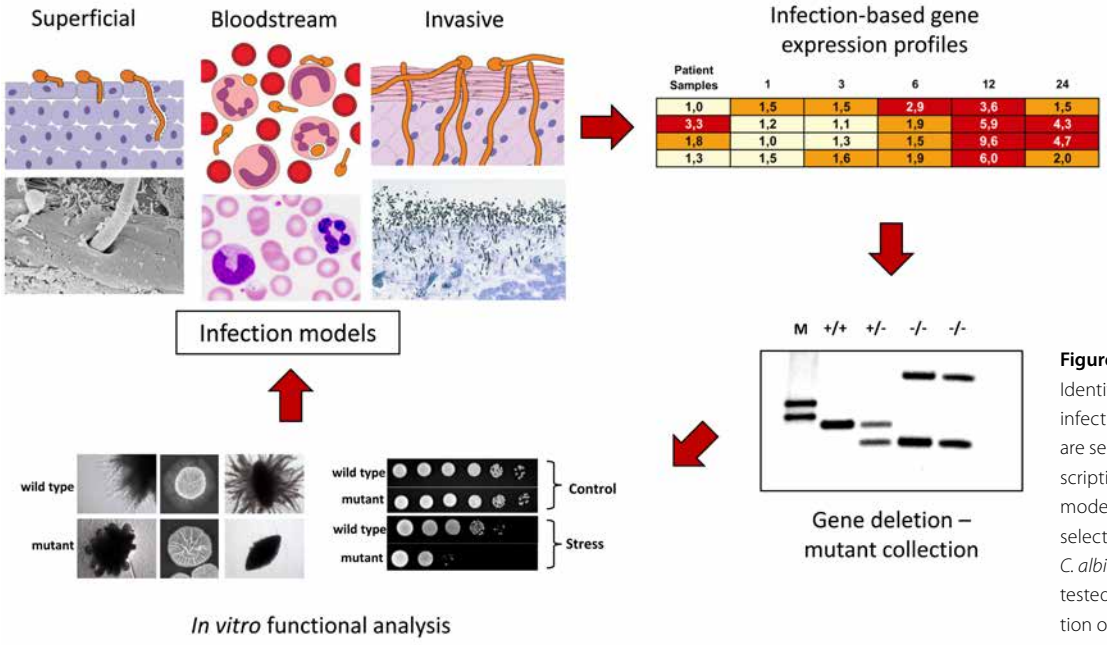

Figure 3
Identification and characterisation of infection-associated genes. Genes are selected based on their transcriptional regulation during distinct models of *Candida albicans* infection; selected genes are then deleted in *C. albicans* and the resultant mutants tested in *vitro to* determine the function of the genes in question; finally, the mutants are tested for pathogenicity in distinct infection models to define novel virulence factors.

cient for disease manifestation, the pathogenicity of *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, invade or 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 were able to assign biological function whilst simultaneously determining the role of the gene product during infection. We have now determined the biological function of a number of these genes and defined their role during infection.

DUR31 mediates extracellular alkalinization and virulence

DUR31 was chosen for further investigation due to its upregulation in samples from HIV-patients with oral candidosis (Zakikhany *et al.*, 2007). A *dur31*Δ/Δ-mutant was investigated for its capacity to damage oral epithelial cells *in vitro* and was found to cause significantly less damage.

In a screen for hyphal formation the *dur31*Δ/Δ-mutant exhibited a defect in foraging hyphae under various conditions, including 10% serum agar, SLAD agar and under embedding conditions.

In a mouse model of hematogenously disseminated candidiasis the mutant was found to be strongly attenuated in virulence. Importantly, *DUR31* was shown to be required for

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uptake of Histatin 5. Moreover, in contrast to the wild type, the *dur31*Δ/Δ-mutant was unable to raise the extracellular environmental pH, indicating that Dur31 lies in the extracellular alkalinization hyphal auto-induction pathway (Mayer *et al.*, 2012, *PLoS Pathogens*, accepted).

A *C. albicans*-unique gene mediates epithelial destruction via controlling hyphal branching patterns

In this part of of the project, we used genome subtraction and functional analysis to identify and characterise genes which are unique to *C. albicans*. Interestingly, each of the *C. albicans* unique genes which we characterised was dispensable for growth *in vitro*, but required for specific stages of host-pathogen interactions. One gene, *PGA16*, was required for epithelial damage by controlling the degree of *C. albicans* hyphal branching. Therefore, the ability of *C. albicans* to generate multiple axes of invasive growth is critical for optimal epithelial destruction.

In summary by functionally characterising a relatively large number of infection-associated genes, we have been able to assign novel functions to previously unknown function genes and identify new virulence factors.

Candida albicans scavenges host zinc via Pra1 during endothelial invasion

Assimilation of essential nutrients by pathogenic microorganisms from their host environment is one of the most fundamental aspects of infection. Host organisms therefore restrict microbial access to certain key nutrients in a process termed nutritional immunity. The mechanisms of iron sequestration, together with the strategies that successful pathogens employ to overcome this restriction have been extensively studied (Skaar *et al.*, 2010). Zinc is the second most abundant trace metal in vertebrates and an important cofactor for around 9% of eukaryotic proteins (Andreini *et al.*, 2009). However, unlike iron, the microbial mechanisms of zinc acquisition are not well understood. Recently, Corbin and co-workers demonstrated that infected mice actively sequester zinc from invading bacteria (Corbin *et al.*, 2008); therefore, the scope of nutritional immunity has expanded beyond iron (Kehl-Fie *et al.*, 2009) and the mechanisms of microbial zinc acquisition represent potential virulence factors.

Our first objective was to determine whether *C. albicans* can acquire zinc from host cells. For this purpose we have developed a new protocol to study *C. albicans* host cell interaction in human umbilical vein endothelial cell (HUVEC) monolayers under conditions in which the only zinc available for *C. albicans* is from the host cells. Zinc-starved yeast cells were incubated either with or without endothelial monolayers in zinc-depleted medium. We performed differential fluorescent staining (Wächtler *et al.*, 2011) to directly visualise and discriminate invading and non-invading fungal elements, we next treated the cells with zinquin, a specific dye which fluorescently labels zinc. We found that the invading hyphal elements bound zinc at significantly higher levels than the non-invading sections (Figure 4). As the only other zinc available to *C. albicans* is from host cells, we reasoned that fungal invasion of the endothelia may facilitate zinc acquisition. We next sought to investigate the molecular bases of host zinc acquisition by *C. albicans*.

By performing an *in silico* analysis of the *C. albicans* secretome for proteins containing zinc binding motifs, we have identified multiple zinc binding domains in Pra1 (pH-regulated antigen 1), a cell wall associated and secreted protein, which is expressed by hyphal cells and which has previously been shown to interact with complement-regulatory proteins and immune cell receptors (Luo *et al.*, 2009). We found that *PRA1* expression was exquisitely sensitive to both chemical- and calprotectin-induced zinc depletion. Moreover, *PRA1* shares its promoter with the high affinity zinc transporter-encoding gene, *ZRT1*. A mutant lacking functional Pra1 (*pra1*Δ) and a mutant lacking functional Zrt1 (*zrt1*Δ) showed growth defects when cultured in zinc limited conditions suggest-

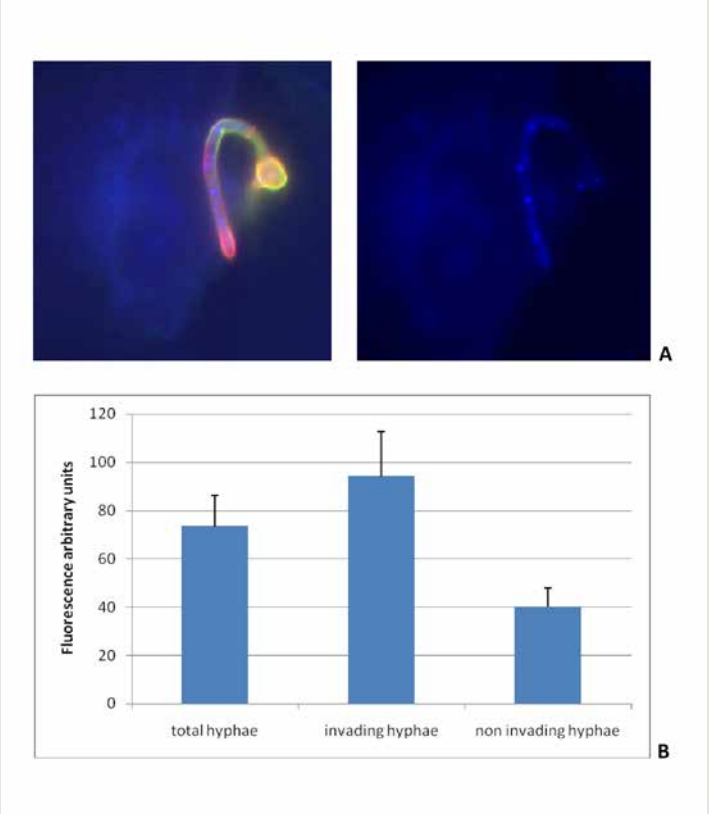

Figure 4
Invasive *C. albicans* hyphae sequester endothelial zinc. **(A)** Endothelial monolayers were infected with wild type *C. albicans* for 3.5 h in zinc-free medium. The invasive sections of *C. albicans* hyphae (in magenta) stain positive for zinquin. **(B)** Quantification of zinquin intensity of *C. albicans* hyphae incubated in zinc-free medium. Intensity of intracellular (invading), extracellular (non invading) and total hyphae was determined.

ing a role for the two protein in zinc acquisition and homeostasis.

Using differential fluorescence microscopy of extracellular and intracellular (invading) fungal cells, combined with specific zinc staining (zinquin), we found that hyphae of the *C. albicans pra1*Δ mutant had significantly decreased abilities to sequester zinc from host cells as compared to wild type cells. Although *pra1*Δ was capable of endothelial invasion, the mutant caused significantly reduced damage of these cells and this effect was rescued by the addition of exogenous Zn. We have also demonstrated, using a colorimetric assay (resorcinol binding assay) that distinguishes zinc transiently and stably bound to proteins, that recombinant Pra1 chelated environmental zinc (in a ratio 1 mol Pra1 : 4 mol zinc). Next, to investigate whether Zrt1 was also required for assimilation of zinc from host cells, we assayed fungal growth with or without endothelial monolayers in zinc-depleted medium. Under control conditions without endothelial cells, wild type, *zrt1*Δ or *pra1*Δ strains grew poorly. In the presence of endothelia, wild type cells formed larger, regularly shaped micro-colonies. In contrast, growth of *zrt1*Δ and *pra1*Δ was not enhanced by the presence of endothelia.

We provided evidence that, in a fashion analogous to siderophore-iron acquisition, the human pathogenic fungus *C. albicans* secretes Pra1, a metal binding protein "zincophore", which sequesters host zinc and is required for damage of host cells in the absence of exogenous zinc. Moreover, Pra1 reassociates with the fungal cell via the syntenically encoded membrane transporter Zrt1. Importantly, we showed that the successful acquisition of this metal is critical for *C. albicans* pathogenicity.

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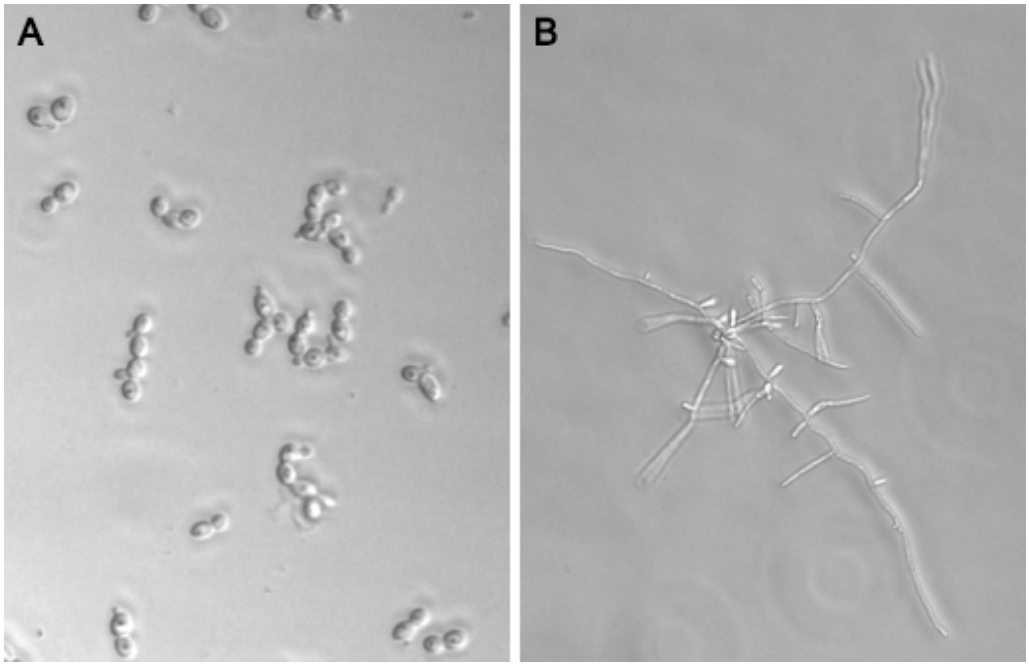

Figure 5
C. albicans is able to grow either as a unicellular budding yeast **(A)** or in filamentous pseudohyphal and hyphal forms **(B)**. The yeast-to-hyphal transition plays an important role during the infection process.

3 Fungal Microevolution

Group leaders: Anja Lüttich, Sascha Brunke, Ilse Jacobsen, Maria Schreiner, Bernhard Hube

Candida albicans and *C. glabrata* are the two most important causes of candidemia – a life-threatening, systemic fungal infection. Factors which determine the virulence potential of *C. albicans* and *C. glabrata* are, for example, metabolic flexibility and site specific expression of metabolic enzymes, mechanisms for nutrient acquisition, such as the uptake of amino acids or iron, and, specific for *C. albicans*, the dimorphism (Figure 5) (Almeida *et al.*, 2008; Barelle *et al.*, 2006; Lorenz *et al.*, 2001; Lo *et al.*, 1997). As commensals and opportunistic pathogens, these yeasts have to be able to adapt to the many different niches inside their human host. We aim to use *in vitro* and *in vivo* forced microevolution experiments, both to determine the ability of these fungi to adapt to host-induced stresses, and to identify novel potential pathogenicity factors. 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 replicate. In the first approach we exposed *C. glabrata* to macrophages in a serial passage setup and followed the genetic, phenotypic, and transcriptional changes induced by constant host-fungus interaction. In a second experiment, we used a mutant of *C. albicans* which is unable to form hyphae, a property which is essential for virulence and escape from phagocytes. Again, the mutant was exposed to macrophages in serial passages and its ability to survive and regain hyphal formation by transcriptional changes *(transcriptional rewiring)* was determined. Finally, we investigated the ability of *C. albicans* to adapt to a specific host niches. In a

murine infection model, we followed the genetic and transcriptional rewiring as well as changes of organotropism of the fungus after serial re-isolation and infection. We expect to find adaptations and mechanisms in both fungi, which may constitute novel, 'hidden' pathogenicity factors.

Microevolution of *C. glabrata* during co-incubation with macrophages

As opposed to *C. albicans*, *C. glabrata* lacks the ability to form hyphae. And while *C. albicans* can use hyphae formation to quickly escape engulfing immune cells such as macrophages, this option is not available to *C. glabrata*, which instead resides inside the phagosome. While much is known about the ability of *C. glabrata* to quickly adapt to antifungal drugs by means of microevolution, we wondered whether an analogous evolutionary adaptation could take place in response to host cells.

Therefore, we continuosly exposed *C. glabrata* yeasts to macrophages in an *in vitro* experiment, isolating the phagocytosed fungal cells and replacing the macrophages daily. Surprisingly, we observed a striking change in fungal morphology after several months in this special form of co-culture: instead of the normal budding yeast form, the fungus started to grow as a chain of tightly attached, elongated cells, resembling the pseudohyphae of *C. albicans*. The macrophages, on the other hand, were now lysed quickly by the fungus growing in this filament-like form. We have performed several infection studies with this strain to characterise its virulence properties, and found that it not only damages macrophages, but also leads to a higher virulence and increased fungal burden in our murine model.

In collaboration with our partners in France, we have resequenced the whole genome of the strain after the microevolution events of our experiment. The actual genetic changes are surprisingly few, and we are now in the process of determining the exact cause for this striking change in phenotype. For that, we seek to combine transcriptome data obtained from wild type and evolved strain, data from phenotypic characterisations, and the genome sequences from both strains.

It seems very likely that these phenomena of microevolution to adapt to the host are not restricted to the *in vitro* situation, but may occur also during long-term exposure of *C. glabrata* to the human host, as it is the case for example during commensalism or in certain types of infection. The ability to express these normally 'hidden' virulence factors may therefore play an important role in the biology and pathogenicity of this fungus.

Microevolution of *Candida albicans* during co-incubation with macrophages

From a collection of mutants, we identified hyphal-deficient strains which cannot escape from macrophages. We chose the *efg1*Δ*/cph1*Δ mutant that fails to form filaments in response to most hyphal-induction conditions, and monitored its adaptation to macrophages during a series of co-culture passages. During this experiment the mutant began to display dramatic phenotypic changes and formed filaments. We tested the filamentation properties of the evolved strain under different filament inducing conditions and during interaction with host cells. In addition, we investigated the virulence potential of the evolved strain compared to the avirulent parental *efg1*Δ*/cph1*Δ mutant in a systemic mouse infection model. Since the phenotype was stable, even in the absence of selective pressure, we conclude that microevolutionary events have led to a bypass of the disrupted Cph1 and Efg1 pathways. To elucidate the genetically stable modifications of the evolved strain we did microarray analysis and analysed the gene expression of different transcription factors by quantitative real-time PCR. Furthermore, in cooperation with the Institute Pasteur (Prof Christoph d'Enfert, Paris, France) we sequenced the evolved and the *efg1*Δ*/cph1*Δ mutant strain.

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In vivo microevolution of *Candida albicans*

Similarly, we did an *in vivo* microevolution experiment to identify factors necessary for survival and tissue destruction in the host. To this date, there exist only few investigations concerning microevolution *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, a group found that *C. albicans* can become attenuated by continued *in vivo* passages through murine spleens (Cheng *et al.*, 2007). Since the kidneys are highly important target organs during invasive candidosis (Mavor *et al.*, 2005), and *C. albicans* can persist in these organs despite a pronounced immune defense, we selected this organ for our microevolution experiments. We propose that during the microevolution experiment *C. albicans* adapts to the kidney and, thus, that the ability to survive in the kidney might be further enhanced. After each passage we analysed the fungal burden and cytokine response in different organs. It turned out that these parameters varied between infected animals but without a clear trend over the passages. Based on the last pool we randomly selected individual fungal clones and tested these *in vitro* for growth, stress resistance and interaction with host cells in order to investigate how homo- or heterogeneous the pool is.

These projects are financed in part by the Jena School for Microbial Communication (JSMC) and the Deutsche Forschungsgemeinschaft DFG Hu 528/17-1 (DACH programme).

4 Pigment production of *Candida glabrata*

Group leaders: Sascha Brunke, Katja Seider, Bernhard Hube

Many pathogenic fungi produce pigments to protect themselves from host defences. Especially melanin, sometimes called the 'fungal armor', can reinforce the fungal cell wall, offer protection against oxygen radicals and antimycotics, and shield the fungus against many other detrimental environmental factors.

A similar, but structurally different, pigment is produced by *C. glabrata* when tryptophan is offered as the sole nitrogen source. In this project, we have elucidated the biosynthetic pathway for this pigment, and have investigated its biological functions for *C. glabrata*.

Using transcriptional analyses, a transposon insertion mutant library, and heterologous protein expression, we have found that the pigment is a by-product of the Ehrlich pathway of aromatic amino acid catabolism. A mutant lacking the tryptophan-upregulated aromatic aminotransferase Aro8 was unable to produce the pigment, while the recombinantly expressed Aro8 protein alone was sufficient for pigment production *in vitro*. In fact, the enzyme catalyses the reaction from tryptophan to indole pyruvate, which itself spontaneously oxidises to form the mainly bisindolic pigment compounds.

The production of the pigment is tightly regulated, as the synthesis is affected by the presence of alternative nitrogen sources, carbon sources, high cell numbers, and cyclic AMP. As soon as the pigment is formed, it is both secreted into the supernatant and attached to the yeast cells. Pigmented *C. glabrata* showed an increased resistance to hydrogen peroxide and had a higher survival rate when exposed to human neutrophils. Additionally, they caused increased damage in a monolayer model of human epithelia, indicating a possible role of pigmentation during interactions with host cells.

This work was 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". Sascha Brunke was financed by the "Studienstiftung des deutschen Volkes".

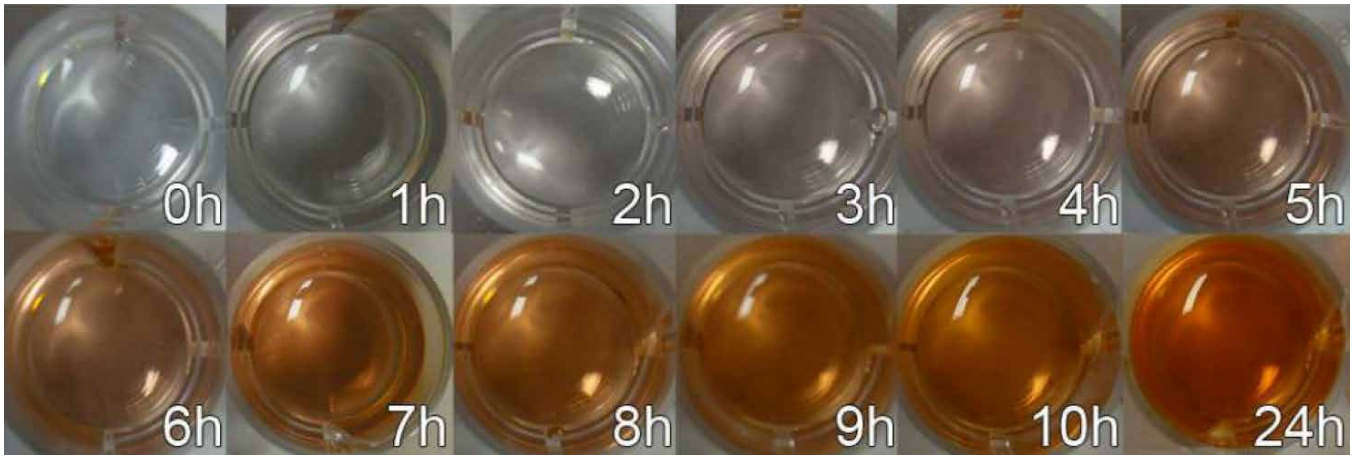

Figure 6
Candida glabrata tryptophan-based pigment production via the Ehrlich pathway.

5 *Candida glabrata* interactions with human cells of the innate immunity

Group Leaders: Katja Seider, Sascha Brunke, Nadja Jablonowski, Bernhard Hube, Lydia Schild

Although we still do not know how *C. glabrata* disseminates in immuno-compromised patients it is clear that the fungus can enter the bloodstream – either through direct penetration from epithelial tissues or due to damage of barriers in the body caused by surgery, polytrauma or drug treatment. Therefore, *C. glabrata* must have developed strategies to counteract or bypass mammalian host defence systems, enabling the fungus to cause systemic disease.

Interestingly, in a systemic mouse model, which was performed in our group, colonisation within organs caused 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* (Jacobsen *et al.*, 2010).

We are focussing on the interactions of *C. glabrata* with phagocytic cells such as macrophages and neutrophils that constitute the first line of defence during infection of the innate immune system. In order to evade or counteract attack by phagocytes, pathogenic yeasts such as *Candida*, *Histoplasma* or *Cryptococcus* species have acquired a repertoire of strategies to survive, colonise and infect the host. These pathogens are able to prevent phagocytosis or to counteract phagocytic activities. Furthermore, these fungi can survive intracellularly within phagocytes and may eventually damage the phagocyte and escape (Seider *et al.*, 2010).

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Survival and replication of *C. glabrata* inside human macrophages

As a main part of the project, we aim to dissect the interaction between *C. glabrata* and human monocyte-derived macrophages (MDMs). We want to identify the strategies that enable *C. glabrata* to survive phagosome biogenesis and antimicrobial activities within human monocyte-derived macrophages. Our data suggest that the normal events of phagosome maturation, which normally lead to the killing of phagocytosed microbes, are actively disrupted by this pathogen.

We began 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 (Seider *et al.*, 2011). We demonstrated that adhesion to and internalisation by macrophages occurs within minutes. Recruitment of early endosomal Ag 1 and lysosomal-associated membrane protein 1 after *C. glabrata* phagocytosis indicates phagosome maturation. The presence of phagosomal membranes tightly surrounding the yeast cells was verified by transmission electron microscopy (in cooperation with Prof Martin Schaller, Tübingen). However, phagosomes containing viable *C. glabrata*, but not heat-killed yeasts, failed to recruit the lysosomal enzyme cathepsin D and were only weakly acidified. A comparison of genome-wide transcriptional profiles of *C. glabrata* cells exposed to media with different pH values to *C. glabrata* cells phagocytosed by macrophages support the view that *C. glabrata* cells within MDMs were not exposed to an acidic pH.

To determine which fungal activities are required for suppression of phagosome maturation and acidification, *C. glabrata* was pretreated with a range of inhibitors to selectively block transcription (thiolutin), translation (cycloheximide), or glycosylation (tunicamycin) prior to incubation with macrophages. Treatment with these different inhibitors did not increase the percentage of acidified phagosomes, suggesting that phagosome manipulation does not rely on transcriptional or translational changes in response to phagocytosis or intact glycosylation. Thus, inhibition of acidification does not require fungal viability, but a heat-sensitive attribute is important for this process.

Despite significant intracellular proliferation of fungal cells, apoptosis or damage of macrophages was not apparent. After prolonged incubation, however, intracellular replication resulted in eventual macrophage lysis, describing a possible escape mechanism of *C. glabrata* from macrophages.

We further showed that production of reactive oxygen species was inhibited by *C. glabrata*. Additionally, with the exception of GM-CSF, levels of pro-inflammatory cytokines IL-1β, IL-6, IL-8, IFNγ and TNF-α were only marginally increased compared to uninfected controls.

Taken together, our results suggest that *C. glabrata* modifies the macrophage phagosome into a non-acidified environment and multiplies until the host cells finally lyse and release the fungi.

Screening of a targeted gene deletion library of *C. glabrata*

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 show an altered survival rate will be further characterised in a number of *in vitro*, *ex vivo* as well as *in vivo* models to investigate the physiological relevance of the lacking gene.

This work is financed in part by the DFG priority programme SPP 1580, Hu 528/16-1.

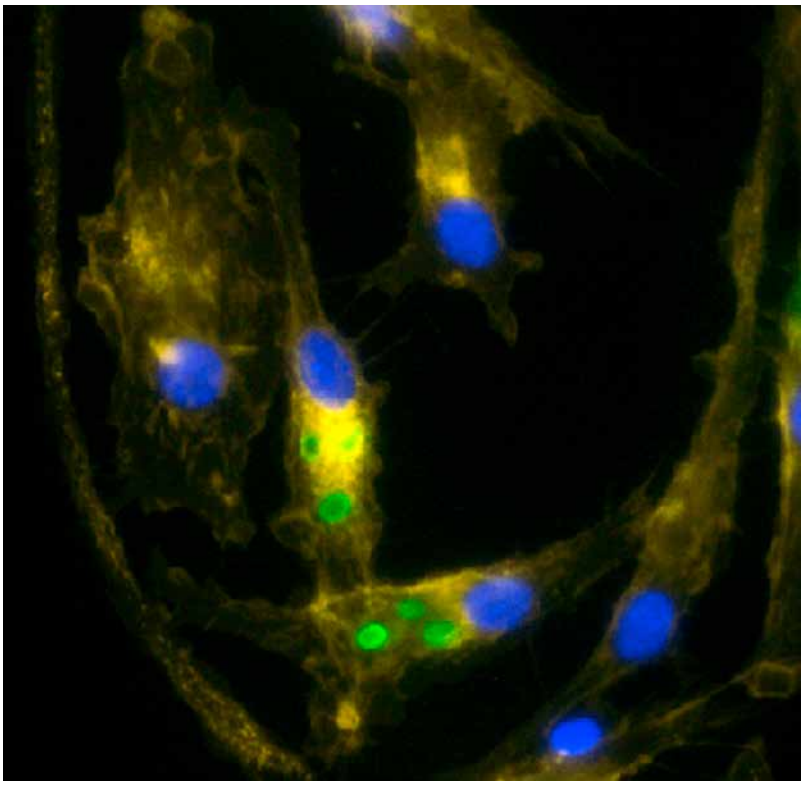

Figure 7
C. glabrata (green) internalised by macrophages (yellow). Blue: macrophage nuclei.

6 Iron sources during infections

Group leaders: Sascha Brunke, Juan Camilo Molina, Bernhard Hube

Iron is the fourth most abundant element on earth, but its biological availability is severely limited. As an important trace element – or micronutrient – it plays central roles in many and diverse biological processes, mainly as a cofactor for a wide range of proteins. Iron toxicity, on the other hand, becomes a problem when its concentration becomes too high. The ability to obtain iron, and to keep this element in homeostasis, therefore is central for both the host and the pathogen, and the struggle for iron is a key element of the infection process: in what is known as 'nutritional immunity', the host restricts the access to iron by sequestering it with different molecules. A successful pathogen, on the other hand, must have mechanisms available to acquire iron even under these limiting conditions (Almeida *et al.*, 2009).

The role of Als3

One of the host's main storage proteins for iron is ferritin, a shell-like intracellular protein of 500 kDa which can contain up to 4,500 Fe^{3+} ions. Overall, about 20 % of the body's iron is bound inside ferritin at any given time. It has been known for a long time that *C. albicans* can use iron-binding host molecules like heme, but ferritin had not been considered a possible iron source during infections in the past.

We have shown that *C. albicans* can in fact bind ferritin and grow both *in vitro* and *in vivo* by using ferritin as an iron source. Using microarray transcriptome data obtained during ferritin-supported growth, we found that the hyphae-associated protein Als3 is upregulated. We created deletion mutants of the *ALS3* gene, and heterologously expressed

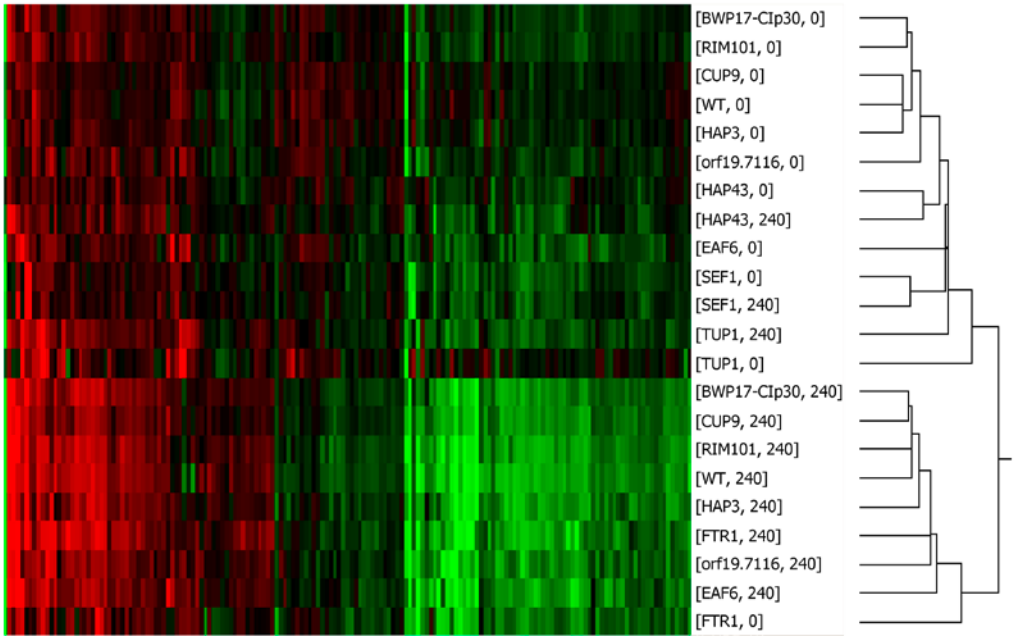

Figure 8
Hierarchical clustering view of the transcriptional profiles of *C. albicans* from a set of mutants under iron depletion conditions.

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Als3 to show that *C. albicans* Als3 is indeed able to bind ferritin. Als3, known as an adhesin with crucial roles in adhesion and invasion, therefore is the *C. albicans* receptor for ferritin (Almeida *et al.*, 2008). With this system, *C. albicans* seems to be able to use the host's own iron storage protein during infections. In our model, the host cell membrane is pierced during infection, and the internally stored ferritin is released and bound by the fungus. This work was the first to show fungal binding of ferritin, and its use as an iron source during *C. albicans* infection.

Modelling the iron regulatory networks of *C. albicans*

To be fully effective, the expression of iron receptors and effector proteins like Als3 or iron transporters must be precisely regulated, depending on the extracellular iron concentrations and the internal fungal iron stores. The regulation of iron homeostasis and the detection of iron starvation conditions are therefore as important to the outcome of an infection as the uptake machinery itself. In an ongoing collaboration with the research group Systems Biology/Bioinformatics at the HKI (Prof Reinhard Guthke, M. Sc. Jörg Linde, Research Group Systems Biology/Bioinformatics), we have created an *in silico* model of the transcriptional response of *C. albicans* to iron depletion. This model is based on transcriptome data from an *in vitro* epithelial infection (Zakikhany *et al.* 2007; Linde *et al.* 2010) and a dedicated time-series transcriptome experiment using microarrays (unpublished data). We used these inferred network models as a basis to predict novel putative interactions in the iron regulatory network of *C. albicans*, and chose central regulators of the network to corrobate and further improve the model.

We have created a set of knock-out mutants of genes with a possible function in iron acquisition, all based on the predictions of the

model. These mutants were then tested for their phenotypes under a wide range of conditions, both iron-related and unrelated, to verify the *in silico* predictions. We were able to find several novel phenotypes in relation to growth under different iron concentrations and with alternative iron sources, but also novel phenotypes which indicate that the iron acquisition network is linked to other cellular processes, such as heat response, uptake of other trace metals, and interaction with the host immune system (Tierney *et al.*, 2012). Additionally, microarrays were used to obtain transcriptional profiles of all these mutants under both iron replete and deplete conditions. Again, we were able to prove previous findings in comparable experiments, but also found novel, and hitherto unknown interactions between the genes under investigation and important transcriptional pathways of the fungus.

Finally, this combined wealth of data comprising transcriptional and phenotype data was then used to feed back into the original model, in an effort to verify and optimise the predictions. This process is known as the "Circle of Systems Biology", and consists of the creation of a model based on previous knowledge, the generation of hypotheses based on this model, and the testing of these hypotheses in the laboratory to create new knowledge, which is integrated in a new round of model generation.

As a result of completing this cycle, we have now obtained a significantly improved network of possible genetic interactions during periods of iron starvation for *C. albicans*. Since iron plays such a pivotal role in nearly all infection scenarios, we will use the novel predicted interactions contained in this network as a basis for our future research on the iron acquisition systems of *C. albicans*. This way, we have started to elucidate aspects of the battle between host and fungus for the essential micronutrient iron.

7 Proteases as virulence factors

Group leaders: Lydia Schild, Antje Heyken, Bernhard Hube

Proteolytic enzymes fulfill 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*.

Ten genes encoding for secreted aspartic proteases, *SAP1-SAP10* have been cloned from *C. albicans*. Saps1-6 have been described to process a variety of host proteins, ranging from human extracellular matrix or surface proteins, such as fibronectin, keratin, collagen and E-cadherin, to host defence molecules like immunoglobulins, cathepsin D, lactoferrin, complement proteins, and pro-inflammatory cytokines (Naglik *et al.* 2003; Gropp *et al.*, 2009).

Inflammatory response induced by *C. albicans* aspartic proteases

In a joint project with the group of Prof Anna Vecchiarelli (Perugia, Italy) we analysed the reaction of human monocytes on recombinant Sap proteases. We showed that Sap proteases are immunostimulatory, inducing a pro-inflammatory cytokine response by human monocytes. Sap1, Sap2, and Sap6 significantly induced interleukin-1β (IL-1β), tumor necrosis factor alpha (TNF-α), and IL-6 production, while Sap3 was able to stimulate the secretion of IL-1β and TNF-α. All Saps tested induced $Ca^{(2+)}$ influx in monocytes. Treatment of Saps with pepstatin A did not have any effect on cytokine secretion, indicating that their stimulatory potential was independent from their proteolytic activity. The induction of inflammatory cytokine production was also independent from protease-

activated receptor (PAR) activation and from the optimal pH for individual Sap activity. The interaction of Saps with monocytes induced Akt activation and phosphorylation of IκBα, which mediates translocation of NF-κB into the nucleus. Overall, these results suggest that individual Sap proteins can induce an inflammatory response and that this phenomenon is independent from the pH of a specific host niche and from Sap enzymatic activity. This inflammatory response is partially dependent on Sap denaturation and is triggered by the Akt/NF-κB activation pathway. Our data suggest a novel, activity-independent aspect of Saps during interactions of *C. albicans* with the host (Pietrella *et al.*, 2010).

Influence of the cell surface-associated proteases Sap9 and Sap10 on the cell biology and pathogenicity of *Candida albicans*

In contrast to Sap family members Sap1-Sap8, Sap9 and Sap10 are cell surface-associated, glycosylated GPI-modified proteins which function in *C. albicans* cell wall integrity and interaction with human epithelial cells. Mutants lacking *SAP9* or *SAP10* genes display defects in cell integrity and cell separation as well as reduced epithelial cell damage during experimental oral infection (Albrecht *et al.*, 2005). These data suggested that Sap9 and Sap10 process fungal cell wall proteins.

To elucidate the specific functions of both proteases in fungal cell wall integrity and virulence, the enzymatic properties of Sap9 and Sap10 and a potential processing of cell surface proteins by these proteases were analysed (Schild *et al.*, 2011). This project was done in collaboration with Dr. Piet de Groot (Amsterdam, The Netherlands) and Prof. Steffen Rupp and Dr. Eckhard Hiller (Stuttgart). Sap9 and Sap10 recombinant proteases were shown to exhibit substantial proteolytic activity over a broad pH range of pH 5-8 with an optimum at near-neutral pH. Inhibition of enzymatic activity by the aspartic protease inhibitor pepstatin A was less efficient for Sap9 and Sap10 in comparison to non surface-associated Sap members.

A systematic evaluation of peptide substrate cleavage by Sap9 and Sap10 showed that both enzymes prefer the processing of peptides containing basic or dibasic residues, dependent on the nature of neighboring amino acids. Both proteases also cleaved at non-basic sites. These enzymatic properties distinguish Sap9 and Sap10 from other *C. albicans* Sap proteases and reveal similarities to *Saccharomyces cerevisiae* yapsins.

Proteomic analyses of a mutant lacking *SAP9* and *SAP10* genes demonstrated that Sap9 and/or Sap10 do not significantly influence secreted or soluble cell wall protein profiles. Recombinant Sap9 or Sap10 proteases, however, cleaved distinct covalently linked cell wall proteins present on isolated cell walls, namely Cht2, Ywp1, Als2, Rhd3, Rbt5, Ecm33, and Pga4. Proteolytic cleavage of the chitinase Cht2 and the glucan-crosslinking protein Pir1 by Sap9 was further shown using hemagglutinin (HA) epitope-tagged versions of both proteins. Deletion of the *SAP9* and *SAP10* genes resulted in a reduction of cell-associated chitinase activity similar to that observed upon deletion of *CHT2,* indicating a direct influence of Sap9 and Sap10 on Cht2 function.

Additionally, we investigated the impact of both proteases on the interaction of *C. albicans* with components of the innate immune system. Alterations on the fungal cell surface elicited by *SAP9* and *SAP10* deletion had no major impact on β-1,3-glucan exposure or phagocytosis and killing of *C. albicans* by human macrophages. In contrast, Sap9 plays an important role for the recognition and killing of *C. albicans* by human neutrophils. In collaboration with Prof Oliver Kurzai (Würzburg, now head of Fungal Septomics, Jena) *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

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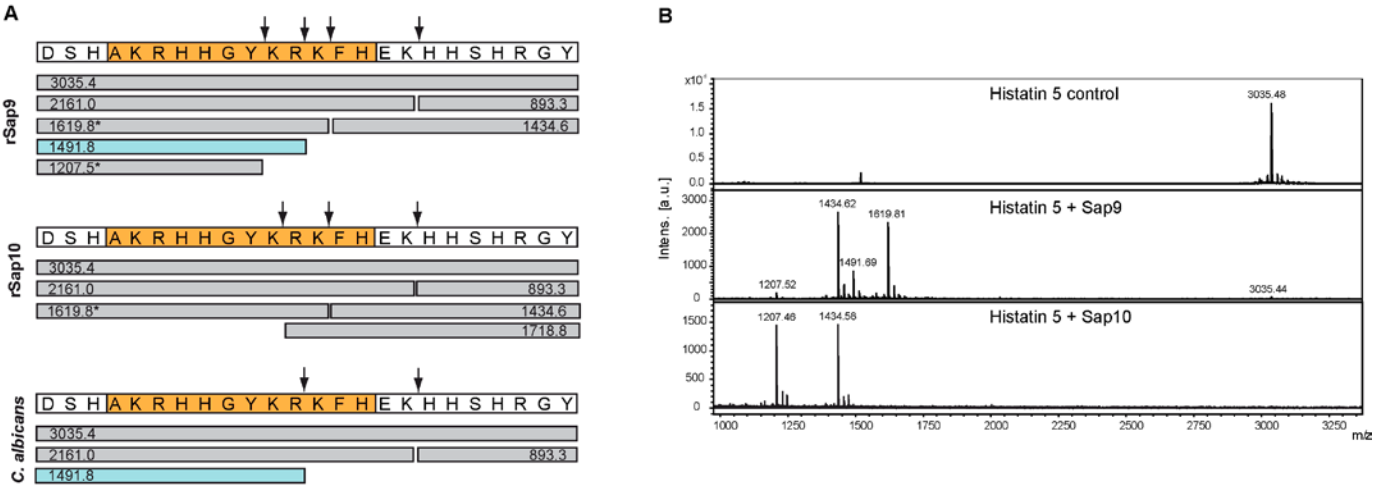

Figure 9
Histatin 5 cleavage by Sap proteases. **(A)** Comparison of cleavage fragments generated by Sap9, Sap10 or *C. albicans* whole cell cleavage. Histatin 5 amino acid sequence with arrows indicating cleavage sites is shown on top. **(B)** MALDI-TOF-MS/MS mass spectra of the peptide mixtures generated after rSap9 and rSap10 histatin 5 digestion.

increased survival of the fungus (Hornbach *et al.*, 2009). In addition, cleavage and inactivation of the human salivary antimicrobial peptide histatin 5 by Sap9 revealed a novel function of Sap9 for proteolysis of host factors as an immune evasion strategy (Meiller *et al.*, 2009). The latter project was done in collaboration with Dr. Mary Ann Jabra-Rizk (Baltimore, USA).

In summary, our data provide insight into the role of Sap9 and Sap10 as yapsin-like aspartic proteases of *C. albicans* with distinct and overlapping functions. The obtained data support the view that both proteases have a regulatory role in modulating specific cell wall functions by proteolytic cleavage of covalently linked cell wall proteins but also digest host peptides. Therefore, Sap9 and Sap10 likely influence *C. albicans*-host interaction by targeting both, fungal cell surface proteins and host components.

This work was financed in part by the Deutsche Forschungsgemeinschaft (DFG, Hu528/14).

8 Interaction of *Candida albicans* with neutrophils

Group leaders: Pedro Miramón, Bernhard Hube

Neutrophils are key phagocytic cells, which play an extremely important role in the innate immune response. They are one of the first types of cells responding to chemoattractans released in sites of infection. Their repertoire of killing mechanisms is vast and extraordinarily effective. As phagocytic cells, they are able to ingest and destroy microbes intracellularly, by exposing them to a mixture of highly toxic substances such as oxidants, proteases and antimicrobial peptides. In addition, neutrophils are also able

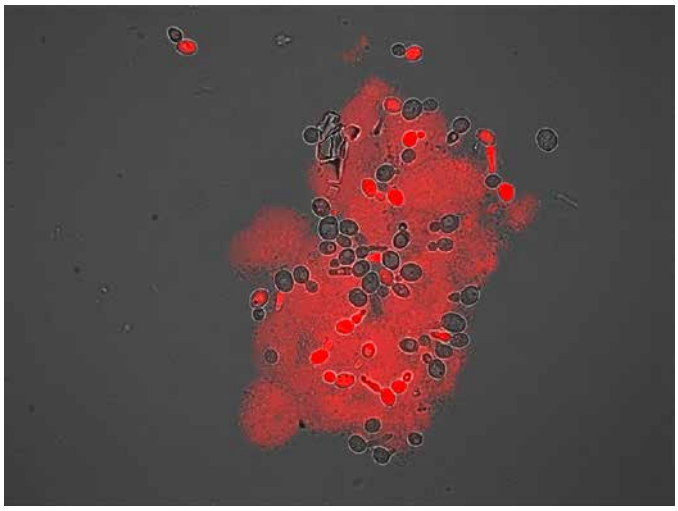

Figure 10
C. albicans cells released from neutrophils and stained with propidium iodide, a red fluorescent dye to assess membrane integrity. Cells with a damaged membrane are permeable to the dye, which intercalates in the DNA.

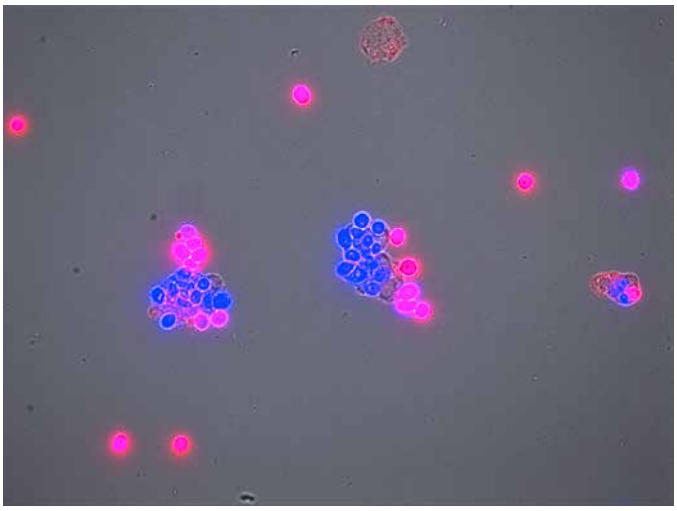

Figure 11
Neutrophils phagocytosing *C. albicans*. In order to distinguish between ingested and non-ingested cells, the sample was differentially stained. Blue fungal cells are inside the neutrophil, while the pink ones are outside.

to exert extracellular inhibition of growth, by releasing the content of their granules to the extracellular milieu. The substances that are released halt the growth of microorganisms, either by causing direct damage, as in the case of oxidants, or by limiting the availability of nutrients. An additional mechanism that neutrophils employ to control the dissemination of microbes is the release of extracellular traps, consisting of DNA fibres decorated with antimicrobial proteins. In addition to clearance, neutrophils are also able to produce chemoattractans, to induce the infiltration of more neutrophils into the site of infection or to regulate their own clearance once they have accomplished their goal.

Neutrophils are the phagocytes with the most potent anti-candidacidal activity. In contrast to *C. albicans* cells phagocytosed by macrophages, *C. albicans* cells are unable to form filaments inside the neutrophil. This means that the fungus is unable to escape from this phagocyte, hence it is effectively killed. However, the key events leading to proper recognition, phagocytosis and killing of *C. albicans* by neutrophils, as well as the response of the fungus to neutrophil-derived extra- and intracellular antimicrobial substances, are still unclear. We have shown that *C. albicans* responds to neutrophils by switching to non-glycolytic metabolism, as well as by upregulating genes coding for ammonium uptake. *C. albicans* also expresses genes coding for detoxifying enzymes, in order to cope with the oxidative and nitrosative insult exerted by the neutrophils (Fradin *et al.*, 2005). We aimed to weigh the contribution of each of these responses in two different subsets of fungal cells: phagocytosed and non-phagocytosed. Thus, we will determine which responses are triggered by intracellular and extracellular activities. These projects are done in collaboration with Prof. Alistair Brown (Aberdeen, UK) and Prof. Oliver Kurzai (Fungal Septomics, Jena).

Neutrophil activation in response to *C. albicans*

C. albicans can exist in a variety of forms, mainly yeast and hypha. It has been demonstrated that neutrophils get activated mostly in response towards the filamentous form, although both types of cells are recognised (Wozniok *et al.*, 2008). A number of different regulators orchestrate the induction and maintenance of filamentation in *C. albicans*, either directly or indirectly. Some of the regulators that influence filamentation of the fungus have, however, a completely different main function. That is the case of Hog1, which is responsible for a proper and robust response of *C. albicans* towards osmotic, oxidative and heavy metal stress (Cheetham *et al.*, 2011). A deletion mutant lacking Hog1 (*hog1*Δ), in addition to an increased sensitivity towards oxidants, also exhibits a misregulated filamentous phenotype. It is therefore highly susceptible to neutrophils, not only because of its fitness defect, but also because of the enhanced filamentation that leads to recognition and killing by neutrophils.

An opposite example is the regulator Cap1. This protein activates the response to oxidative stress (Zhang *et al.*, 2000), and does not have any obvious link to filamentation. However, the deletion mutant *cap1*Δ, although able to filament, has a slower filamentation rate. Strikingly, despite being exquisitely sensitive to oxidants *in vitro*, this mutant is poorly recognised and killed by neutrophils.

One way to assess neutrophil activation is by measuring the production of oxidants or reactive oxygen species. They are produced as a consequence of the respiratory burst in neutrophils, performed by the NADPH oxidase complex, which releases superoxide radicals further converted into hydrogen peroxide and other highly toxic derivatives. Reactive oxygen species are only produced in response to stimuli, such as bacteria, fungi, endotoxins or chemicals, and they are released to the extracellular environment or into the phagolysosome. *C. albicans* has several enzymes that detoxify oxidants. Particularly important are three surface-associated enzymes that detoxify superoxide, named superoxide dismutases. Deletion mutants lacking the genes coding for *SOD4*, *SOD5* and *SOD6* accumulate oxidants, leading to decreased survival. Of these, the isoenzyme 5 (Sod5), seems to be the most important for detoxification, which is also the case when facing macrophages (Frohner *et al.*, 2009). Other detoxifying enzymes have different contributions when *C. albicans* is challenged with neutrophils. For example, a catalase seems to be dispensable, but glutathione peroxidases may have a discrete contribution in oxidants scavenging, without being essential for normal survival.

In addition to oxidants, neutrophils also produce nitric oxide (NO). This substance also has detrimental activities against microbes, either by acting directly on its targets, or by combining with hydrogen peroxide or halides, rendering peroxynitrite or hypochlorous acid, respectively. *C. albicans* has a nitric oxide dioxigenase, encoded by *YHB1.* Yhb1 transforms NO into nitrate, a non-toxic substance. Expression of *YHB1* increases in response to NO producing agents (Hromatka *et al.*, 2005) and also during contact with neutrophils. We have shown that Yhb1 plays an important role in survival, demonstrating that *C. albicans* responds to neutrophil-imposed nitrosative stress.

Single cell profiling and mutants analysis

We also use specific promoter driven GFP expression to assess the transcriptional behaviour 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 (*MEP2*), oxidative stress (*CTA1*, *SOD5*, *TTR1*, *TRX1*) and nitrosative stress (*YHB1*, *SSU1*). To differentiate between internalised and non-phagocytosed cells, we use a counterstaining which labels only cells outside the neutrophils. To determine the importance of the gene products encoded by these genes, we have also investigated the

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susceptibility of deletion mutants to killing by neutrophils.

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9 Infection models

Group leaders: Silvia Slesiona, Katharina Große, Volker Schwartze, Ilse Jacobsen

The use of suitable models for the analysis of virulence and pathogenicity is an important part of 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. 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. We are interested in pathogenesis mechanisms – the complex interplay between host and fungus and the factors which affect the ultimate outcome of infection. In 2010 to 2011, Ilse Jacobsen, Silvia Slesiona, Katharina Große, Volker Schwartze, Birgit Weber and Ursula Stöckel were involved in the projects outlined below.

Murine infection models for *Candida glabrata*

To study pathogenesis of *C. glabrata*, we developed a model of systemic infection in mice. In contrast to life-threatening infections in immunocompromised patients, mice infected with *C. glabrata* do not develop disease upon systemic challenge, even with high infection doses. We could show that leukopenia, but not treatment with corticosteroids, leads to increased fungal burdens. However, even immunocompetent mice are not capable of clearing infections within four weeks. Strikingly, only a very moderate immune response was detectable after infection, indicating that *C. glabrata* has successfully developed immune evasion strategies enabling it to survive, disseminate, and persist within mammalian hosts.

Furthermore, we could show that histidine, leucine, or tryptophan auxotrophy, as well as a combination of these auxotrophies, does not influence survival of *C. glabrata* in immunocompetent mice. Thus, *C. glabrata* histidine, leucine, or tryptophan auxotrophic strains are suitable for the generation of knockout mutants for *in vivo* studies (Jacobsen *et al.*, 2010).

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

Infection models for *Aspergillus terreus*

Aspergillus terreus is emerging as a causative agent of life-threatening invasive aspergillosis. Prognosis for affected patients is often worse than for *A. fumigatus* infections. In collaboration with Dr. Matthias Brock (Head of Junior Research Group Microbial Biochemistry and Physiology, Jena) we developed two murine infection models to study *A. terreus* mediated disease. In leukopenic mice the outcome of invasive aspergillosis was similar to that described for *A. fumigatus,* but 10^2 and 10^3 fold higher conidia concentrations were required for 100 % lethality. In corticosteroid-treated mice, only 50 % mortality was observed, although bioluminescence imaging revealed transient disease in all infected animals. In surviving animals, we observed persistence of ungerminated but viable conidia. Cytokine levels in these mice were comparable to uninfected controls. In contrast to *A. fumigatus* infections, all mice infected with *A. terreus* developed fatty liver degeneration, suggesting the production of toxic secondary metabolites. Thus, at least in mice, persistence and subclinical liver dam-

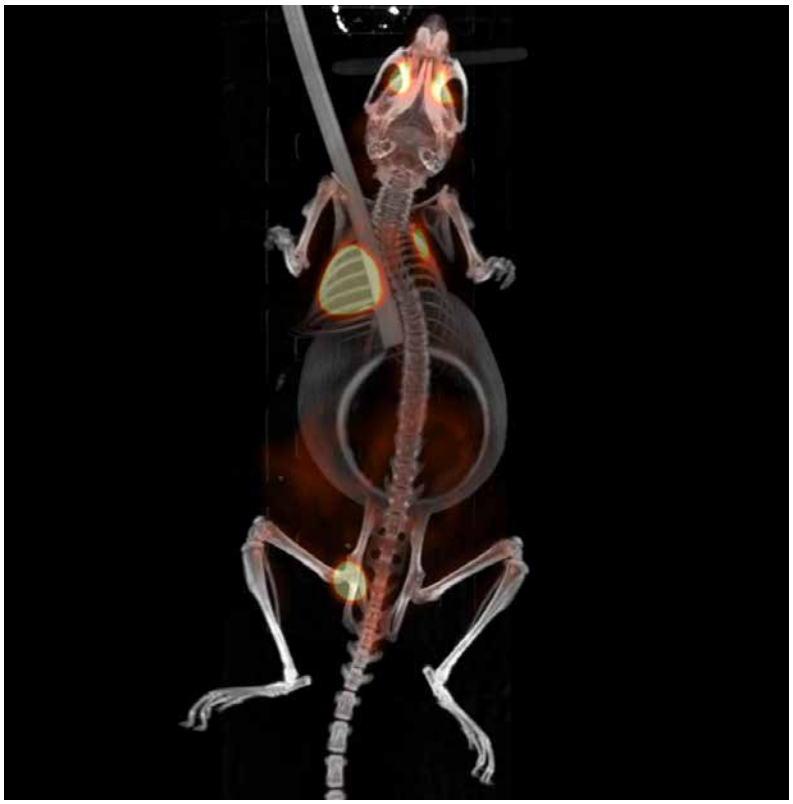

Figure 12
Mouse immunosuppressed with cortisone acetate and FDG accumulation in the lung after *Aspergillus fumigatus* infection.

age are unique features of *A. terreus* infections (Slesiona *et al.*, 2012).

The murine data suggested that the initial steps of disease establishment might be fundamentally different between *A. terreus* and *A. fumigatus*. Since alveolar macrophages represent one of the first phagocytes facing inhaled conidia, we compared the interaction of *A. terreus* and *A. fumigatus* conidia with alveolar macrophages. *A. terreus* conidia were phagocytosed more rapidly than *A. fumigatus* conidia, possibly due to higher exposure of β-1,3-glucan and galactomannan on the fungal surface. In agreement, blocking of dectin-1 and mannose receptors significantly reduced phagocytosis of *A. terreus*, but had only a moderate effect on phagocytosis of *A. fumigatus*. Once phagocytosed, and in contrast to *A. fumigatus*, *A. terreus* did not inhibit acidification of phagolysosomes, but remained viable without signs of germination both *in vitro* and in immunocompetent mice. The inability of *A. terreus* to germinate and pierce macrophages resulted in significantly lower cytotoxicity compared to *A. fumigatus*. Blocking phagolysosome acidification by the v-ATPase inhibitor bafilomycin increased *A. terreus* germination rates and cytotoxicity. Recombinant expression of the *A. nidulans* wA naphthopyrone synthase, a homologue of *A. fumigatus* PksP, inhibited phagolysosome acidification and resulted in increased germination, macrophage damage and virulence in corticosteroid-treated mice. In summary, we have demonstrated that *A. terreus* and *A. fumigatus* have evolved significantly different strategies to survive the attack of host immune cells. While *A. fumigatus* prevents phagocytosis and phagolysosome acidification and escapes from macrophages by germination, *A. terreus* is rapidly phagocytosed, but conidia show long-term persistence in macrophages even in immunocompetent hosts (Slesiona *et al.*, 2012).

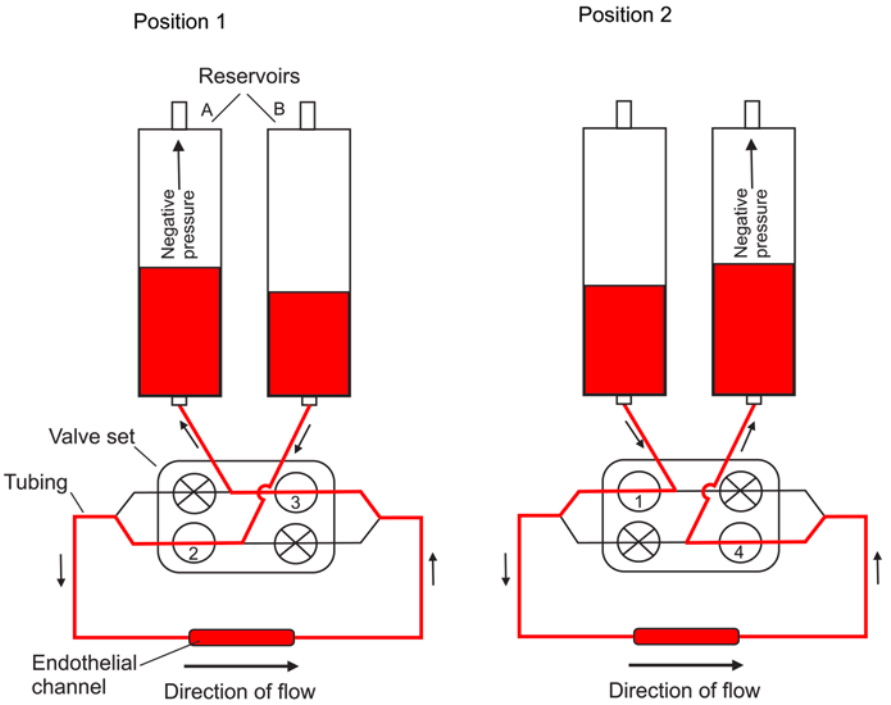

Figure 13
The circulatory model. Schematic representation of the circulatory model. Air pressure in the reservoirs and opening/closing of the valve set are controlled via an air pump and computer software. Note that in both position 1 and position 2 unidirectional flow through an endothelial channel is maintained.

This work is financed in part by the Deutsche Forschungsgemeinschaft (DFG, Br-2216/4-1).

Chicken embryos as an alternative infection model for pathogenic fungi

In 2010 and 2011, we have developed and characterised embryonated chicken eggs as alternative infection models to study the virulence of *Candida* spp., *Aspergillus* spp. and zygomycetes of the genus *Lichtheimia*.

Embryonated eggs as infection models for *A. fumigatus* and *A. terreus* were developed in collaboration with Dr. Matthias Brock (Head of Junior Research Group Microbial Biochemistry and Physiology, Jena) (Jacobsen *et al.*, 2010; Slesiona *et al.*, 2012). The outcome of infections in the egg model is dose and age dependent and highly reproducible. We show that the age of the embryos affects the susceptibility to infection and that increased resistance coincides with altered chemokine production after infection. The progress of disease in the model can be monitored via egg survival and histology. Based on pathological analyses, we hypothesise that invasion of embryonic membranes and blood vessels leads to embryonic death. Defined deletion mutant strains previously shown to be fully virulent or partially or strongly attenuated in a mouse model of bronchopulmonary aspergillosis showed comparable degrees of attenuation in the egg model. Addition of nutrients restored the reduced virulence of a mutant lacking a biosynthetic gene, and variations of the infectious route can be used to further analyse the role of distinct genes in our model.

Furthermore, we used this model successfully in collaboration with the institute of Veterinary Pathology, Berlin, to study the virulence potential of different *A. fumigatus* strains isolated from pulmonary aspergillosis in stork chicks (Olias *et al.*, 2011).

During infections with *Candida albicans* and *C. glabrata*, the developing stage of the immune system of the host significantly influenced susceptibility: With increasing age, embryos became more resistant and mounted a more balanced immune response, characterised by lower induction of proinflammatory cytokines and increased transcription of regulatory cytokines, suggesting that immunopathology contributes to pathogenesis. While many aspects of the chicken embryo response resembled murine infections, we also observed significant differences: In contrast to systemic infections in mice, IL-10 had a beneficial effect in chicken embryos. IL-22 and IL-17A were only upregulated after the peak mortality in the chicken embryo model occurred; thus, the role of the Th17 response in this model remains unclear. Abscess formation occurs frequently in murine models, whereas the avian response was dominated by granuloma formation. Pathogenicity of the majority of 15 tested *C. albicans* deletion strains was comparable to the virulence in mouse models and reduced virulence was associated with significantly lower transcription of proinflammatory cytokines. However, fungal burden did not correlate with virulence and for few mutants like *bcr1*Δ and *tec1*Δ different outcomes in survival compared to murine infections were observed. *C. albicans* strains locked in the yeast stage disseminated significantly more often from the CAM into the embryo, supporting the hypothesis that the yeast morphology is responsible for dissemination in systemic infections. These data suggest that the pathogenesis of *C. albicans* infections in the chicken embryo model resembles systemic murine infections but also differs in some aspects. Despite its limitations, it represents a useful alternative tool to pre-screen *C. albicans* strains to select strains for subsequent testing in murine models.

Together with the group of Dr. Kerstin Voigt (Jena Microbial Resource Collection, Friedrich Schiller University, Jena), we furthermore established the CAM model to investigate the virulence potential of *Lichtheimia* species, a genus causing mucormysoses in animals and humans. Using this model we could demonstrate that the virulence potential of clinically relevant species is higher than that of other species and that clinical and environmental isolates of the same species have comparable virulence potentials.

This work is financed in part by the Federal Ministry of Education and Research (BMBF).

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) and bioluminescence/fluorescence imaging 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 visualise 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 visualise both inflammation and fungal burden in the same animal by using fluorescent probes to detect immune cells and bioluminescent fungal cells.

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This work is financed in part by the Federal Ministry of Education and Research (BMBF).

An *in vitro* model of the circulatory system

The translocation of *Candida albicans* from the blood stream to internal organs represents a key stage of disseminated candidosis. However, for such translocation to occur, the fungus must first adhere to the endothelial lining of blood vessels under conditions of physiological pressure and flow.

The ibidi pump system (ibidi – Integrated BioDiagnostics, Germany) is used for perfusing *C. albicans* cells through an endothelial channel. The general features of the system are shown in Figure 13. The system comprises a fluidic unit, which can be placed in an incubator, and an air pressure pump – both of which are computer-controlled. The fluidic unit holds the perfusion set, which consists of two reservoirs connected to opposite ends of the endothelial channel via silicone tubing. The silicone tubes from each reservoir branch above the valve set and rejoin with a branch of the opposing tube below the valve set. Within the valve set, each of the four silicone tube branches can be independently opened and closed. This set up allows continuous unidirectional flow of medium through the endothelial channel: in position one, valves 2 and 3 are open and negative pressure from the air pump is generated in reservoir A; in position two, valves 1 and 4 are open and negative pressure is generated in reservoir B. Simultaneous switching of air pressure and valve sets between position one and two generates medium movement through the tubing (arrows) and maintains continuous unidirectional flow through the endothelial channel.

With this set-up, *C. albicans* cells can be perfused through the endothelial channel at pressures similar to those found in human capillaries.

Using this system we have demonstrated that a key stage of hyphal formation, under the control of the hypha-specific G1 cyclin Hgc1, is necessary for *C. albicans* adhesion to endothelium under conditions of physiological flow (Wilson *et al.*, 2010).

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Identification and characterisation of virulence associated genes during oral infections with *Candida albicans*
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Deutsche Forschungsgemeinschaft
SPP 1580: Intracellular Compartments as Places of Pathogen-Host Interaction
Phagosom Survival and proliferation of human pathogenic *Candida* species within phagocytes
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DACH Evolution – Microevolution of pathogenic yeasts during interactions with the host immune system
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Cell surface associated secreted aspartic proteases (SAPs) of *Candida albicans*
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Selected publications

Dalle F, **Wächtler B**, L'Ollivier C, Holland G, Bannert N, **Wilson D**, Labruère C, Bonnin A, **Hube B** (2010) Cellular interactions of *Candida albicans* with human oral epithelial cells and enterocytes. *Cell Microbiol* 12, 248-271.

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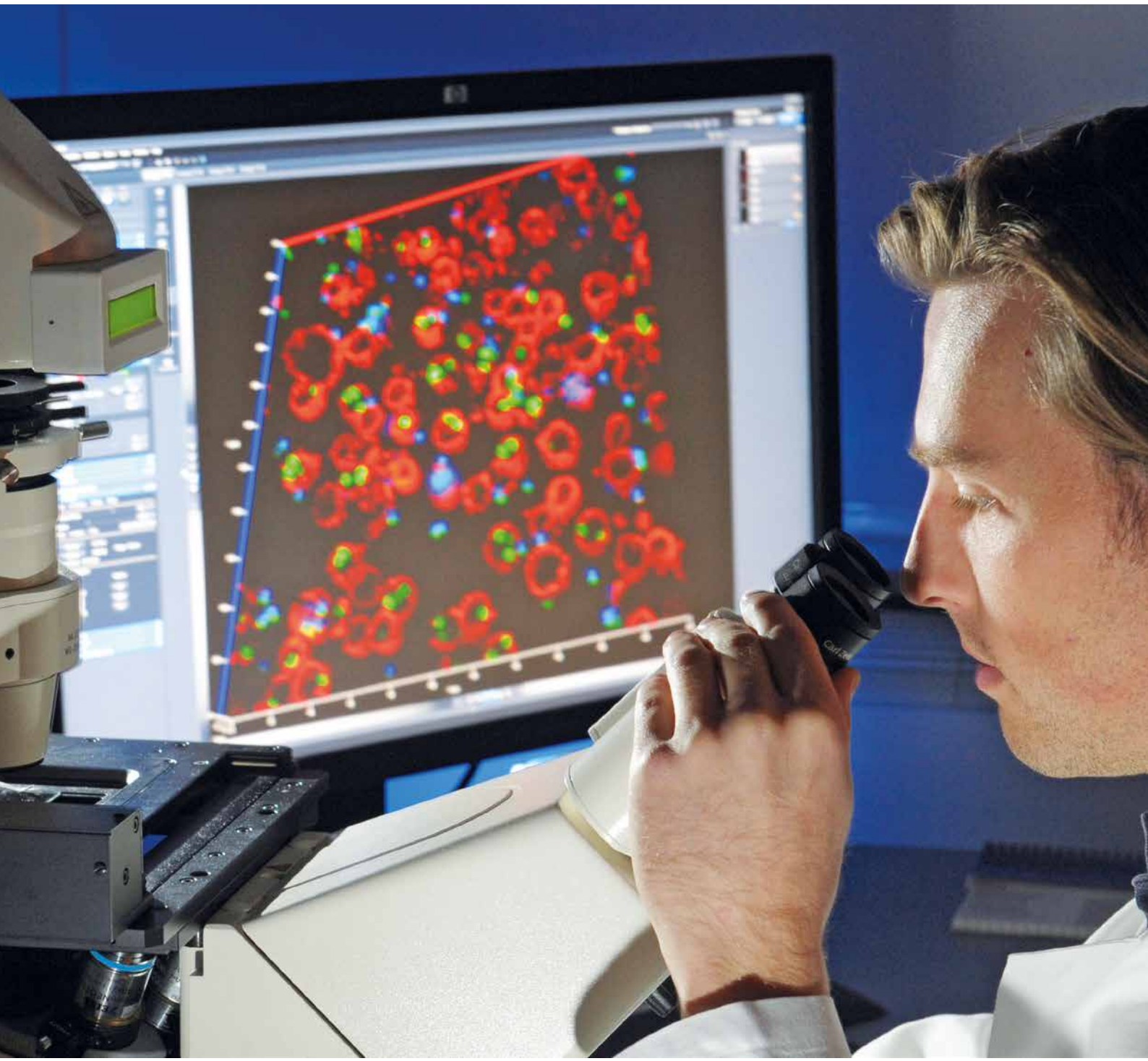
Wächtler B, Wilson D, Hube B (2011) *Candida albicans* adhesion, invasion and damage of vaginal epithelial cells: stage-specific inhibition by clotrimazole and bifonazole. *Antimicrob Agents Chemother* 55, 4436-4439.



Department of Molecular and Applied Microbiology

Department Molecular and Applied Microbiology

The Department of Molecular and Applied Microbiology (MAM) headed by Prof. Axel A. Brakhage covers the two main research areas of the HKI – natural product research and infection biology – by applying modern methods from molecular biology and biochemistry. The production and regulation of natural products such as mycotoxins and antibiotics in the mold fungi *Aspergillus nidulans* and *Aspergillus fumigatus* is one field of research. In the area of infection biology, the department investigates *A. fumigatus* as the most important airborne human pathogenic fungus. This fungus can cause life-threatening infections in immunocompromised patients, e.g. after bone marrow transplants. Furthermore, research in the department also includes work on skin fungi, so-called dermatophytes, which are the main cause for fungal infections in humans. In the following, these research topics will be introduced briefly:

1. Microbial communication and activation of silent fungal gene clusters for secondary metabolites
   Fungi are producing numerous secondary metabolites, natural products, which often are biologically active. Well known examples include the antibiotic penicillin and the mycotoxin aflatoxin. It is assumed that these compounds play a crucial role in microbial communication. The Department MAM addresses the exciting question which environmental conditions and which types of interactions with other microorganisms are required for the production of fungal secondary metabolites.

INTRODUCTION | EINLEITUNG

Head:
Prof. Dr. Axel A. Brakhage

Die Abteilung Molekulare und Angewandte Mikrobiologie bearbeitet unter der Leitung von Prof. Axel A. Brakhage mit modernen molekularbiologischen und biochemischen Methoden beide Themenschwerpunkte des HKI: die Naturstoff-Forschung sowie die Infektionsbiologie. Einerseits untersucht die Abteilung die Produktion und Regulation von Naturstoffen, beispielsweise Mykotoxine und Antibiotika, bei den Schimmelpilzarten *Aspergillus nidulans* und *Aspergillus fumigatus*, andererseits erforscht sie *A. fumigatus* als den wichtigsten, über die Luft verbreiteten humanpathogenen Pilz. Dieser kann lebensbedrohliche Infektionen bei Patienten mit stark geschwächtem Immunsystem auslösen, z.B. bei Patienten mit Knochenmark-Transplantation. Des Weiteren schließt die Forschung der Abteilung auch Hautpilze, die sogenannten Dermatophyten, mit ein. Sie sind die häufigsten Verursacher von Pilzinfektionen beim Menschen. Einleitend sollen die Forschungsfelder hier kurz vorgestellt werden:

1. Mikrobielle Kommunikation und Aktivierung stiller, pilzlicher Sekundärmetabolismus-Gencluster
   Pilze produzieren eine Vielzahl sekundärer Stoffwechselprodukte, Naturstoffe, die häufig eine biologische Wirkung besitzen. Bekannte Vertreter sind z.B. das Antibiotikum Penicillin oder das Pilzgift Aflatoxin. Es wird vermutet, dass diese Verbindungen eine wichtige Rolle bei der mikrobiellen Kommunikation spielen. In der Abteilung MAM wird die spannende Frage untersucht, unter welchen Bedingungen und bei welcher Form der Interaktion mit anderen Mikroorganismen pilzliche Sekundärmetabolite gebil-

A special focus is on the activation of certain densly packed DNA regions that contain genes for natural product synthesis.

2. Pathobiology of *Aspergillus fumigatus*
   Around 80 % of all invasive mycoses by mold fungi in immunocompromised patients are caused by *A. fumigatus*. Research focuses on the identification of properties which allow *A. fumigatus* to sporulate in patients with reduced immune responses, to proliferate and to spread throughout the body. To answer this question, modern methods from molecular biology, genomics, transcriptomics, proteomics, system biology and infection research are used. In collaboration with partners from academic research and industry, research to improve diagnostics and therapy is carried out.

3. Eukaryotic transcription factors
   Another focus of research in the department lies on the mechanistic details of the regulation of secondary metabolite gene clusters, the iron balance and the redox state of a fungal cell. Methods to study protein/protein and protein/DNA interactions, such as surface plasmon resonance and micro calorimetry, are used. X-ray crystallography of protein complexes and protein/DNA complexes is carried out in collaboration with other laboratories.

det werden. Hier steht insbesondere die Aktivierung bestimmter, dicht gepackter DNA-Regionen, auf denen die Gene für die Synthese von Naturstoffen liegen, im Vordergrund der Forschung.

2. Pathobiologie von *Aspergillus fumigatus*
   A. fumigatus ist für ca. 80 % aller invasiven, durch Schimmelpilzarten hervorgerufenen Pilzinfektionen in immunsupprimierten Patienten verantwortlich. Im Fokus der Forschung steht die Frage, welche Eigenschaften es *A. fumigatus* ermöglichen, bei Patienten mit gestörter Immunantwort auszukeimen, sich zu vermehren und im Körper zu verbreiten. Um diese Frage zu beantworten, werden moderne Methoden der Molekularbiologie, Genomik, Transkriptomik, Proteomik und Systembiologie und Infektionsforschung angewandt. In Zusammenarbeit mit Partnern der akademischen Forschung und Industrie wird auch an der Verbesserung der Diagnose und Therapie geforscht.

3. Eukaryotische Transkriptionsfaktoren
   Ein weiterer Schwerpunkt der Abteilung befasst sich mit den mechanistischen Details der Regulation von pilzlichen Sekundärmetabolitgenclustern, des Eisenhaushalts und des Redoxstatus einer Pilzzelle. Zum Einsatz kommen Methoden, die sich zur Untersuchung von Protein/Protein und Protein/DNA-Wechselwirkungen eignen, z.B. die Technik der Oberflächen-Plasmon-Resonanz und Mikrokalorimetrie. In Zusammenarbeit mit anderen Gruppen werden Röntgenstrukturanalysen von Proteinkomplexen und Protein/DNA-Komplexen durchgeführt.

1 Proteomics of filamentous fungi

Group Leaders: Olaf Kniemeyer,
Axel A. Brakhage

The Department of Molecular and Applied Microbiology is devoted to research on the biology and pathogenicity of the two molds *Aspergillus nidulans* and *Aspergillus fumigatus* as well as the skin fungus *Arthroderma benhamiae*. The species *Aspergillus nidulans* is one of the best studied model organisms for filamentous fungi. *Aspergillus fumigatus* is the main cause for airborne opportunistic fungal infections in humans including invasive infections, while the dermatophyte *Arthroderma benhamiae* is known for its ability to cause superficial skin infections in humans and animals. The recent releases of their genome sequences, the entirety of the fungus' hereditary information, have paved the way for global studies on gene expression and protein biosynthesis in these filamentous fungi. Our aim in the "Fungal Proteomics" group is to gain a detailed insight into the stress response and pathogenicity of the two *Aspergillus* species and the dermatophyte *A. benhamiae* on the level of proteins. Proteins are the active agents within a cell and participate in many important biological processes, such as forming the cell's scaffold or catalysing numerous biochemical reactions. In principal, two methods are applied to investigate an organism's complete set of proteins, the proteome: First of all 2D-gel electrophoresis and second liquid-chromatography coupled with mass spectrometry. Both methods are used in our lab and in the following paragraphs we report some exemplary research projects of the research group "Fungal Proteomics".

Proteome profiling and functional classification of intracellular proteins from dormant spores of *Aspergillus fumigatus*.
As a tool for further studies on the biology and immunology of *Aspergillus fumigatus* infection we established a 2D reference map for *A. fumigatus* spore proteins. Proteome maps provide information about all the proteins, which are present in a specific organism at a specific time and which are accessible to 2D-gel electrophoresis. This technique is based on the separation of proteins by charge and size in a gel matrix and allows to separate between 500-1000 different protein variants from microorganisms. Visible protein spots can be excised from the gel matrix, cleaved in smaller peptides and analysed and identified by mass spectrometry.

We have gained special interest in conidia, the asexually produced spores, since they are the fungal entity having the initial contact with the host during the infections. By MALDI-TOF mass spectrometry, we identified a total number of 449 different proteins. Enzymes involved in reactive oxygen species (ROS) detoxification, pigment biosynthesis, and conidial rodlet layer formation were highly abundant in *A. fumigatus* spores and most probably account for their enormous stress resistance. Moreover, we could show that enzymes for rapid reactivation of protein biosynthesis and metabolic processes are preserved in resting conidia, which therefore feature the potential to immediately respond to an environmental stimulus by germination. Such a rapid response is also important at the initial phase of infection. The generated data lay the foundations for further proteomic analyses and a better understanding of fungal pathogenesis and immunogenic activity.

Figure 1
Mycelium of *Aspergillus fumigatus* cultivated under (A) normal oxic and (B) hypoxic growth conditions in a chemostat.

Analysis of the *Aspergillus fumigatus* response to low atmospheric oxygen levels (hypoxia)

The ability to adapt to low oxygen levels seems to be an important trait of *A. fumigatus* to be able to cause infections in humans with impaired immune system. Therefore, together with co-operation partners from the Medical University of Innsbruck and the Leibniz University of Hannover we aimed at obtaining a comprehensive overview about this process on the protein level. To ensure highly reproducible growth conditions we established an oxygen-controlled chemostat, that means a bioreactor to which fresh medium is continuously added, while on the same time culture liquid is continuously removed. Such a system allowed us to control the growth rate of *A. fumigatus* under both normoxic (atmospheric oxygen concentration) and hypoxic conditions (Figure 1). After 10 days of cultivation proteins from the fungal mycelium were extracted and separated by two-dimensional gel electrophoresis. Proteins spots with change in abundance due to the hypoxic conditions were excised from the gel and identified by mass spectrometry. We identified 117 different proteins with an altered abundance under hypoxic in comparison to normoxic conditions. Hypoxia induced an increased activity of enzymes involved in primary energy metabolism and the biosynthesis of amino acids, the building blocks of proteins. Consistently, the cellular contents of components of metalloproteins, which are involved in respiration and redox reactions increased upon hypoxia, such as the trace elements iron, copper and zinc. Surprisingly, hypoxia induced also the biosynthesis of the secondary metabolite pseurotin A. Secondary metabolites are small, organic molecules which are not directly involved in the primary metabolism of a cell, that is in the generation of energy and the biosynthesis of cell building blocks. Fungi are well known for their production of toxins and compounds

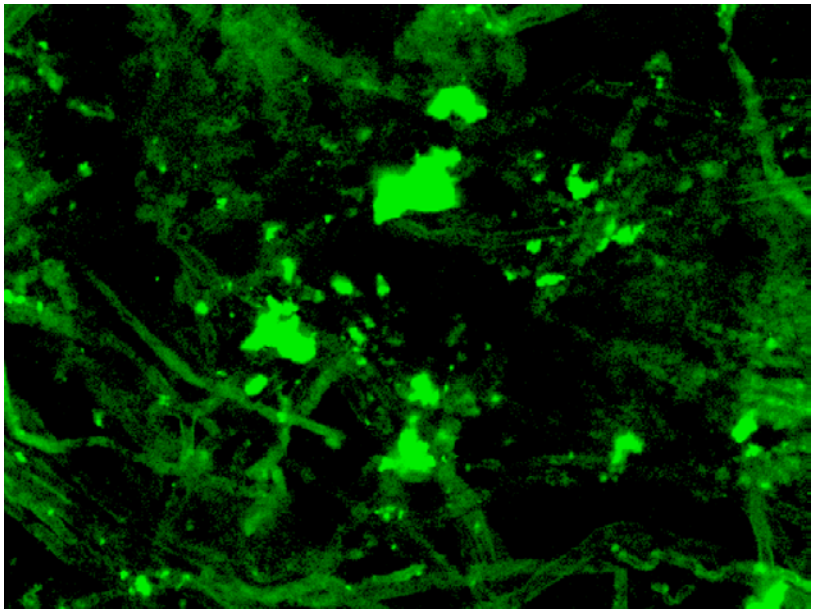


Figure 2
Mycelium of *Aspergillus fumigatus* in a biofilm embedded in an extracellular matrix. Mycelium was stained with the fluorescence stain Alexafluor 488 (photo from Marc Seidler, Heidelberg).

with antimicrobial activity. The observed and so far not reported stimulation of the biosynthesis of a secondary metabolite by oxygen depletion may also affect the survival of *A. fumigatus* in hypoxic niches of the human host. However, the biological effect of pseurotin A on human cells has not been elucidated in detail yet. Several studies revealed an immunosuppressive and cytotoxic effect of pseurotin A on immune cells, e. g., it was shown that pseurotin A reduced the production of allergic antibodies. Another outcome of our study was that a link between hypoxia and the generation of nitrosative stress may exist in *A. fumigatus*. In further experiments we would like to gain further insight into the complex metabolic changes of *A. fumigatus* during growth under hypoxic conditions.

Functional genomic profiling of *Aspergillus fumigatus* biofilms

Bacteria and yeasts often grow as biofilms in the environment, that is an aggregate of microorganisms in which cells form extracellular polymeric substances and adhere to surfaces. Biofilms are often resistant to antibiotics and the activity of immune cells. Therefore biofilms have a significant impact on the pathogenesis of microorganisms. Just recently, biofilm formation in filamentous fungi has gained increased interest. It appeared that also *A. fumigatus* can grow in multicellular communities by the formation of a hyphal network encased in an extracellular matrix. Such a biofilm exhibits reduced antifungal drug susceptibility. This intrinsic drug resistance of *A. fumigats* biofilms has most probably also an impact on the efficiency of antifungal therapy. To understand the molecular details of biofilm formation in *A. fumigatus*, we compared the proteome and transcriptome of planktonic- and biofilm-grown *A. fumigatus* mycelium after 24 and 48 h (Figure 2).

The biofilm- and time-dependent regulation of many proteins and genes involved in pri-

mary metabolism suggested that young biofilms after 24 h growth show high metabolic activity, while during the matured biofilm phase, metabolic activity seems to be reduced. This is consistent with findings from yeast and bacterial biofilm cells, which also show reduced metabolic rates during the mature stage. In contrast, genes and proteins involved in the biosynthesis of secondary metabolites were significantly upregulated. In particular, proteins of the gliotoxin biosynthesis secondary metabolite gene cluster were induced in biofilm cultures. As already described in the previous section, the synthesis of secondary metabolites is often triggered by environmental factors and growth conditions. The enhanced production of gliotoxin by *in vitro* formed biofilms reported by our group in collaboration with the group of Frank-Michael Müller from Heidelberg (now Itzehoe) may also play a significant role under *in vivo* conditions during the infections. Gliotoxin acts on numerous cells of the immune system and suppresses their activity. It is interesting to speculate that this mycotoxin may confer protection from the host immune system to *A. fumigatus* and also enable its survival and persistence in chronic lung infections such as aspergilloma, a localised *Aspergillus* infection in immunocompetent patients. Interestingly, typical biofilm structures were observed in individuals with aspergilloma and most likely gliotoxin is produced in this group of patients. However, a direct proof for this fact is still missing.

2 Virulence of *Aspergillus fumigatus*

Group Leaders: Thorsten Heinekamp, Axel A. Brakhage

Tyrosine degradation and pyomelanin formation in *A. fumigatus*

A. fumigatus is able to produce the brown pigment pyomelanin by degradation of L-tyrosine. Pyomelanin was shown to protect the fungus against reactive oxygen intermediates as well as cell wall disturbing compounds and is therefore assumed to protect against the attack of host immune effector cells during the infection process. Several genes for tyrosine degradation and pyomelanin formation are organised in a cluster in the genome of *A. fumigatus*.

To further analyse tyrosine degradation and a possible role of pyomelanin in virulence the function of two not yet characterised genes of the cluster, i.e., *hmgX* and *hmgR*, was analysed. Generation of corresponding gene deletion mutants and reconstituted strains revealed that *hmgX* and *hmgR* are essential for tyrosine degradation. Both mutants, Δ*hmgX* and Δ*hmgR*, are not able to use tyrosine as sole carbon or nitrogen source and show impaired pyomelanin production. HmgR harbours a Zn(II)2Cys6-DNA binding domain and analyses of the steady state mRNA levels revealed that HmgR acts as a transcriptional activator for the genes of the tyrosine degradation cluster. Consistently, an HmgR-eGFP fusion protein localises in the nucleus of *A. fumigatus* cells. By contrast, HmgX localises in the cytoplasm and does not contribute to regulation of gene transcription. HPLC analyses showed that HmgX is crucial for the conversion of *p*-hydroxyphenylpyruvate to homogentisic acid, the main intermediate in pyomelanin formation. HmgX is supposed to function as an accessory factor to mediate specific activity of the *p*-hydroxyphenylpyruvate dioxygenase HppD. Remarkably, the ability to degrade tyrosine and to form pyomelanin is dispensable for virulence of *A. fumigatus* in a murine infection model.

Interaction of *A. fumigatus* with the host endocytosis pathway

The interaction of *A. fumigatus* with the host's immune system represents a key process to understand pathogenicity (Figure 3). To eliminate invading microorganisms, they need to be efficiently phagocytosed and located in acidified phagolysosomes. However, *A. fumigatus* is able to manipulate the formation of functional phagolysosomes. To elucidate the nature of this interference, the interaction of *Aspergillus* conidia with different types of immune cells was characterised

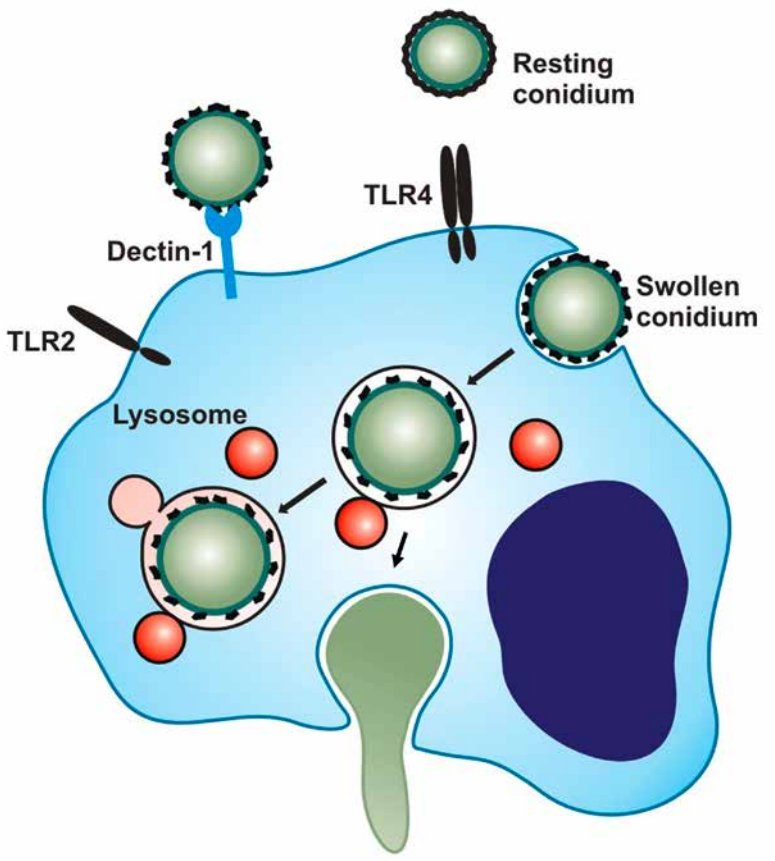


Figure 3
Model for recognition and phagocytosis of *A. fumigatus* conidia by macrophages.
After recognition by pattern recognition receptors (e.g. Dectin-1), swollen conidia are phagocytosed and fusion of the conidium-containing phagosome with lysosomal vesicles forms the phagolysosome. However, *A. fumigatus* conidia are able to escape killing in the phagolysosome and to germinate.

by means of confocal laser scanning microscopy. In contrast to pigmentless *pksP* mutant conidia of *A. fumigatus*, the grey-green wild-type conidia inhibit the acidification of phagolysosomes of alveolar macrophages, monocyte-derived macrophages and human neutrophil granulocytes (Figure 4). Studies with melanin ghosts indicate that the inhibitory effect of wild-type conidia is due to their dihydroxynaphthalene (DHN)-melanin covering the conidia, whereas the hydrophobin RodA rodlet layer plays no role in this process. This is also supported by the observation that *pksP* conidia still exhibit the RodA hydrophobin layer, as shown by scanning electron microscopy. Mutants defective in different steps of the DHN-melanin biosynthesis showed stronger inhibition than *pksP* mutant conidia but lower inhibition than wild-type conidia. Moreover, *A. fumigatus* and *Aspergillus flavus* led to a stronger inhibition of phagolysosomal acidification than *Aspergillus nidulans* and *Aspergillus terreus*. The vATPase was identified as a potential target for *A. fumigatus* based on the finding that addition of bafilomycin which inhibits vATPase, led to complete inhibition of the acidification whereas the fusion of lysosomes with conidia-containing phagosomes was not affected. These data indicate that a certain type of DHN-melanin that is different in various *Aspergillus* species is required for maximal inhibition of phagolysosomal acidification (Figure 4).

G protein-coupled receptors of *A. fumigatus*

In order to survive in diverse habitats, such as compost piles and human lungs, *A. fumigatus* 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. Two putative G-protein-coupled receptors,

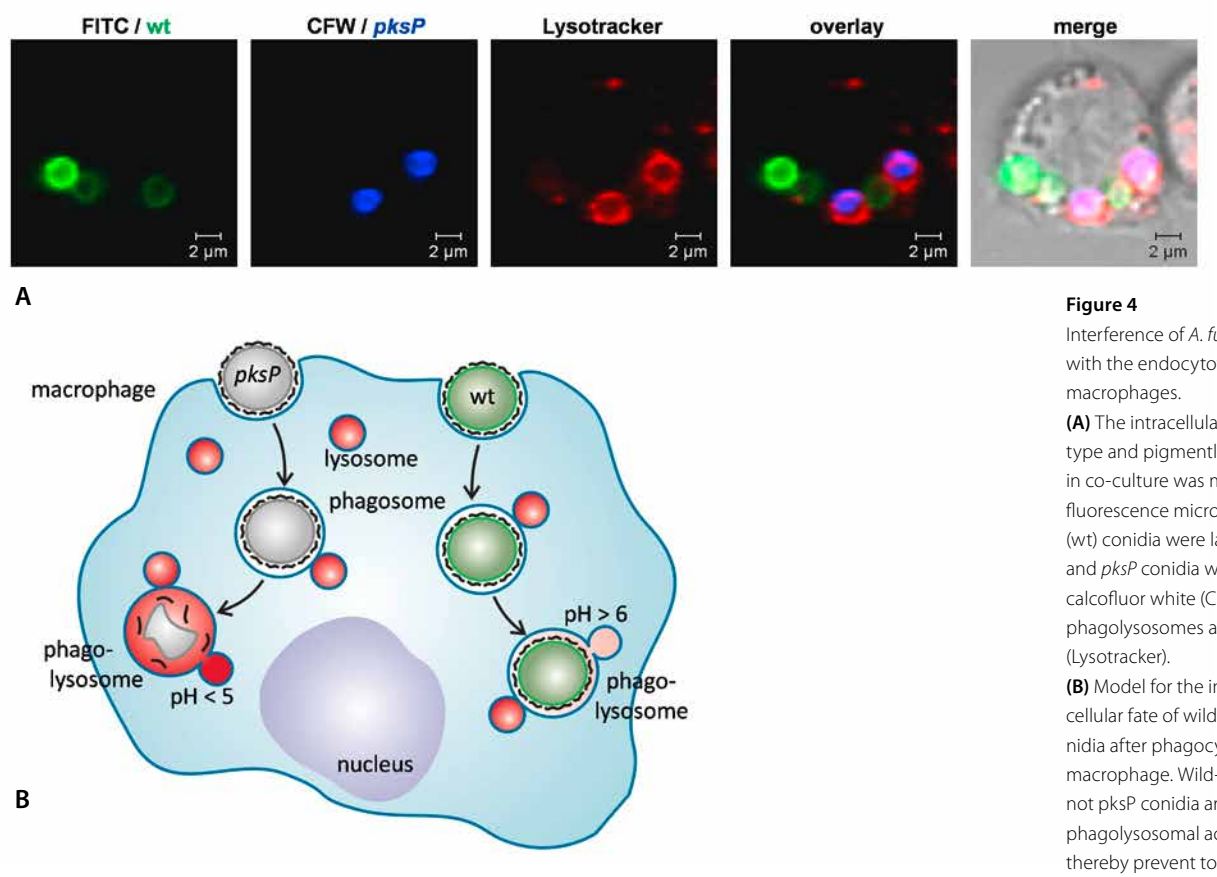


Figure 4
Interference of *A. fumigatus* conidia with the endocytotic pathway of macrophages.
(A) The intracellular fate of wild-type and pigmentless *pksP*-conidia in co-culture was monitored by fluorescence microscopy. Wild-type (wt) conidia were labeled with FITC, and *pksP* conidia were labeled with calcofluor white (CFW). Acidified phagolysosomes are stained red (Lysotracker).
(B) Model for the independent intracellular fate of wild-type and *pksP* conidia after phagocytosis by the same macrophage. Wild-type conidia but not pksP conidia are able to inhibit phagolysosomal acidification and thereby prevent to be degraded by lytic proteins.

GprC and GprD, were characterised with respect to their cellular functions. Deletion of the corresponding genes results in drastic growth defects as reduced hyphal extension, retarded germination, and elevated levels of branching of hyphae. The sensitivity of the mutant strains toward environmental stress caused by reactive oxygen intermediates is increased and the mutants display an attenuation of virulence in a murine infection model. Both mutants, especially the Δ*gprC* strain, exhibits increased tolerance toward cyclosporine, an inhibitor of the calcineurin signal transduction pathway. Transcriptome analyses indicated that in both the *gprC* and *gprD* deletion mutants, transcripts of primary metabolism genes were less abundant, whereas transcription of several secondary metabolism gene clusters was upregulated. Taken together, our data suggest that the receptors are involved in integrating and processing stress signals via modulation of the calcineurin pathway.

Secreted proteins of *A. fumigatus*

Surface-associated and secreted proteins represent primarily exposed components of *A. fumigatus* during host infection. Although several secreted proteins are known to be involved in defence mechanisms or immune evasion there was only limited knowledge about the composition of the secretome and about molecular functions of particular proteins. To identify secreted proteins potentially essential for virulence, the core secretome of *A. fumigatus* grown in minimal medium was determined. Two-dimensional gel electrophoretic separation and subsequent MALDI-TOF-MS/MS analyses resulted in the identification of 64 different proteins. Additionally, secretome analyses of *A. fumigatus* utilising elastin, collagen or keratin as main carbon and nitrogen source were performed. Thereby, the alkaline serine protease Alp1 was identified as the most abundant protein and hence presumably represents an important protease during host infection. Interest-

ingly, the Asp-hemolysin (Asp-HS), which belongs to the protein family of aegerolysins and which was often suggested to be involved in fungal virulence, was present in the secretome under all growth conditions tested. In addition, a second, non-secreted protein with an aegerolysin domain annotated as Asp-hemolysin-like (HS-like) protein was found to be encoded in the genome of *A. fumigatus*. Generation and analysis of Asp-HS and HS-like deletion strains revealed no differences in phenotype compared to the corresponding wild-type strain. Furthermore, hemolysis and cytotoxicity was not altered in both single-deletion and double-deletion mutants lacking both aegerolysin genes. All mutant strains showed no attenuation in virulence in a mouse infection model for invasive pulmonary aspergillosis.

Regulation of stress response and secondary metabolite biosynthesis by the MAP kinase MpkA

The cell wall of *A. fumigatus* has been studied intensively as a potential target for development of effective antifungal agents. A major role in maintaining cell wall integrity is played by the mitogen-activated protein kinase (MAPK) MpkA. To gain a comprehensive insight into this central signal transduction pathway transcriptome analysis of the Δ*mpkA* mutant under cell wall stress conditions was performed. Besides genes involved in cell wall re-modelling, protection against ROS and biosynthesis of secondary metabolites such as gliotoxin and pseurotin A, also genes involved in siderophore biosynthesis were regulated by MpkA. Consistently, iron starvation triggers phosphorylation and thus activation of MpkA. Localisation studies of an eGFP-MpkA fusion protein by fluorescence microscopy indicated that MpkA accumulates in the nucleus under iron depletion. The measurement of amino acid pools and of the pools of polyamines indicated that arginine was continuously converted into ornithine to fuel the siderophore pool in the Δ*mpkA* mutant strain. Therefore, MpkA is responsible for fine-tuning the balance between stress response and energy consuming cellular processes.

3 Transcription factors and signal transduction / Protein-protein interactions

Group Leaders: Peter Hortschansky, Axel A. Brakhage

Biosynthesis and function of gliotoxin in *Aspergillus fumigatus*

Gliotoxin is a virulence factor of the human pathogen *Aspergillus fumigatus*, the leading cause of invasive aspergillosis, and the prototype of a class of epidithiodioxopiperazines (ETPs) that are produced by several fungal species. Its toxicity is mediated by the exceptional transannular disulfide bridge of the ETP scaffold. Various studies have focused on the action of gliotoxin on different cell types, showing that most of the activities mainly depend on an intact disulfide bridge within the molecule. Notably, gliotoxin induces apoptosis, inhibits the proteasome and angiogenesis, to mention some of its effects on host cells.

All genes involved in gliotoxin biosynthesis are organised in a cluster comprising 13 genes within the *A. fumigatus* genome (Figure 5). All gliotoxin biosynthesis genes are under major control of the Zn_2Cys_6 binuclear cluster transcription factor GliZ. The *gliZ* gene is localized within the cluster as well, and consequently, its deletion resulted in loss of gliotoxin production, whereas over-expression significantly increased the gliotoxin level. The largest gene of the cluster (*gliP*) encodes for a non-ribosomal peptide synthetase (NRPS) that mediates the synthesis of the diketopiperazine (DKP) core.

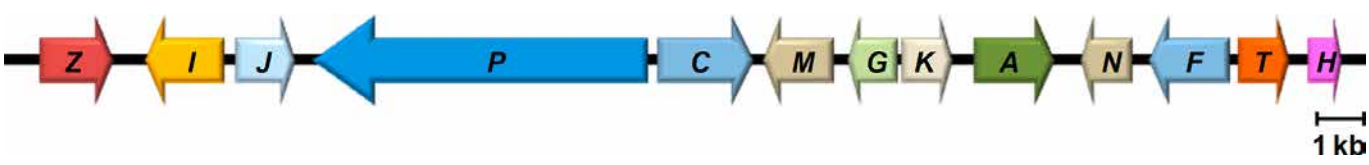


Figure 5
Gliotoxin biosynthesis gene cluster of *A. fumigatus*. The cluster is composed of the following genes: *gliZ*, Zn_2Cys_6 binuclear cluster transcription factor; *gliI*, 1-aminocyclopropane-1-carboxylate synthase; *gliJ*, a dipeptidase; *gliP*, a two-module NRPS; *gliC*, gliF, two cytochrome P450 monooxygenases; *gliM*, O-methyltransferase; *gliG*, a glutathione-S-transferase; gliK, a hypothetical protein; *gliA*, a major facilitator type transporter; *gliN*, a methyltransferase; *gliT*, a gliotoxin oxidase; *gliH*, a conserved hypothetical protein.

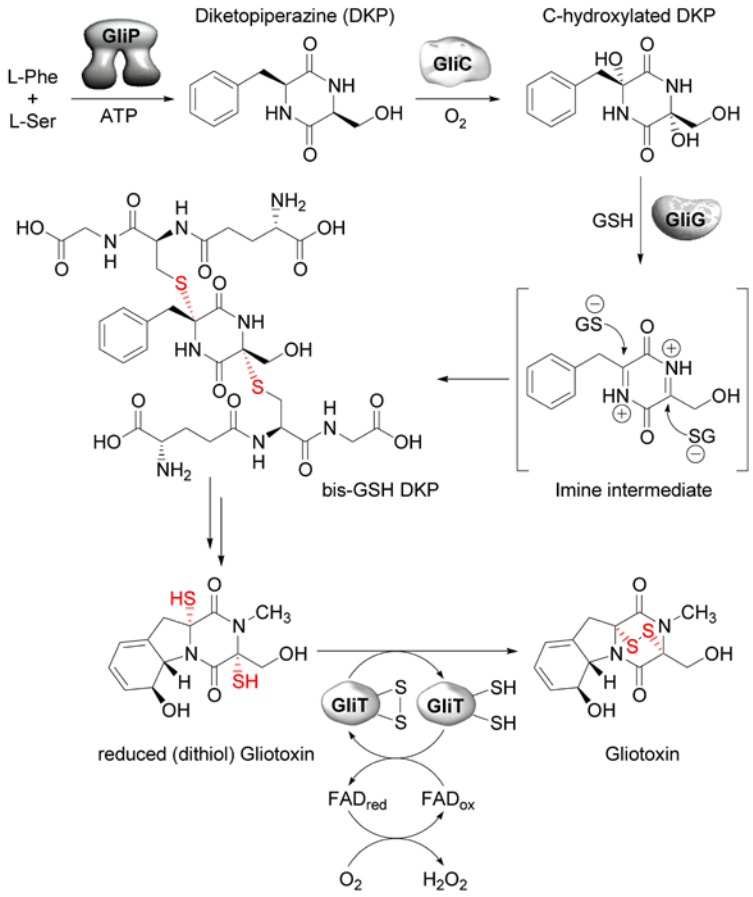


Figure 6
Proposed biosynthetic pathway for gliotoxin biosynthesis in *A. fumigatus*.

Whereas models for gliotoxin biosynthesis and their evolution have been deduced from genomic data, the enzymatic mechanisms and pathway intermediates have remained enigmatic until recently. In collaboration with the Department of Biomolecular Chemistry, we were able to elucidate two of the most intriguing steps in the gliotoxin pathway: (i) introduction of sulfur into the diketopiperazine precursor and; (ii) formation of the disulfide bond within gliotoxin.

C-S bond formation by the GliG glutathione S-transferase, and a cytochrome P450 monooxygenase, GliC

Bioinformatic analysis of the gliotoxin biosynthesis gene cluster revealed that the *gliG* gene could code for a glutathione S-transferase (GST). However, despite their widespread occurrence, GSTs have not been implicated in fungal biosynthetic pathways. Through the targeted knockouts of the *gliG* and *gliC* genes and subsequent metabolic profiling we have now sufficient evidence for a scenario where first a C-hydroxylated DKP derivative is generated from the DKP precursor by GliC, a putative cytochrome P450 monooxygenase. Subsequently, elimination of water would generate an imine intermediate that is attacked by the nucleophilic cysteine thiolate residues of two glutathione (GSH) moieties. Furthermore, we found that the C-hydroxylated DKP is the precursor of gliotoxin, as it was consumed in a GliG *in vitro* assay with formation of a novel sulfur-containing bis-GSH DKP adduct (Figure 2). This mode of C-S bond formation is likely to be significant for a broad range of fungi, as our phylogenetic analysis revealed that genes coding for GliG homologues are widespread in the genomes of ETP producers.

Formation of the disulfide bridge by the gliotoxin oxidase GliT

The *gliT* gene has been annotated as a pyridine dinucleotide dependent oxidoreductase that is likely involved in the formation of the

disulphide bridge within gliotoxin. However, biochemical evidence for this assumption was lacking until recently. By combining genetic, biochemical, and chemical analyses, we found that GliT catalyses the formation of gliotoxin from the corresponding reduced dithiol precursor. *In vitro* studies using purified GliT revealed that the FAD-dependent, homodimeric enzyme utilizes molecular oxygen as terminal electron acceptor with concomitant formation of hydrogen peroxide (Figure 6).

Phylogenetic analyses suggest that GliT is the first characterised member of a completely new class of biosynthetic enzymes producing intramolecular sulfur bridges in natural products using oxygen as co-substrate and FAD as essential co-factor. Beside the crucial function of GliT in gliotoxin biosynthesis itself, it mediates self-resistance of *A. fumigatus* against gliotoxin. This resistance relies on the ability of GliT to keep gliotoxin in the oxidized state, which avoids generation of reactive oxygen species (ROS) and production of protein conjugates. The intact disulfide bridge might be also a prerequisite for the gliotoxin excretion from the fungal cell. The widely distributed nature of disulfide bond-forming enzymes and their crucial role for some organisms place them into the focus of research based on the mechanisms and features of these enzymes and their potential role as anti-fungal target.

4 Regulation of fungal secondary metabolism genes

Group Leaders: Volker Schroeckh, Axel A. Brakhage

Bacteria-induced natural product formation in the fungus *Aspergillus nidulans* requires Saga/Ada-mediated histone acetylation

Microorganisms have an enormous potential to produce low molecular-weight molecules. Moreover, the increasing number of fungal and bacterial genome sequences indicated a large underestimation of their genetic capability to produce such compounds. For example, the important model fungus *Aspergillus nidulans* harbours 28 putative polyketide synthase (PKS) and 24 nonribosomal peptide synthetase (NRPS) gene clusters, giving this fungus the potential to produce at least 52 different secondary metabolites. Therefore, an important question is how many of these potentially useful compounds are actually overlooked, mainly due to the fact that most of these gene clusters are silent under common laboratory conditions. To make this untapped reservoir accessible, a number of strategies have been established during the last years comprising special growth conditions, genetic engineering or epigenetic modulation. A challenging question is to identify the true function of these compounds in the habitat to develop rational strategies for the activation of their biosyntheses. In this context, we recently discovered that the intimate physical interaction of *A. nidulans* with *Streptomyces rapamycinicus*, a soil-dwelling bacterium, led to the activation of a silent polyketide synthase (PKS) gene cluster encoding for the archetypal polyketide orsellinic acid, its derivative lecanoric acid, and the cathepsin K inhibitors F-9775A and F-9775B. This finding indicated that communication between microorganisms can indeed play a key role in activating silent gene clusters. Since *A. nidulans* is well-suited to study various fundamental biological questions, this opened up the opportunity to shed light on the molecular mechanisms that form the basis of communication between the fungus and the bacterium.

Previously, it was reported that chromatin modifications contribute to the regulation of gene clusters. However, none of these studies addressed the question of whether this regulation is important in a natural setting of interacting microorganisms. In an integrative study we discovered that the streptomycete *S. rapamycinicus* triggers modification of fungal histones. Deletion analysis of 36 of 40 acetyltransferases, including histone acetyltransferases (HATs) of *A. nidulans*,

demonstrated that the Saga/Ada complex containing the HAT GcnE and the AdaB protein is required for induction of the orsellinic acid gene cluster by the bacterium. We also showed that Saga/Ada plays a major role for specific induction of other biosynthesis gene clusters, such as sterigmatocystin, terrequinone, and penicillin. Chromatin immunoprecipitation showed that the Saga/Ada-dependent increase of histone 3 acetylation at lysine 9 and 14 occurs during interaction of fungus and bacterium. In summary, we identified the Saga/Ada complex, a conserved chromatin modifying complex in eukaryotes, as an essential part of the streptomycete induced secondary metabolite gene cluster activation in *A. nidulans*. Furthermore, we confirmed that Saga/Ada is a central regulator in secondary metabolism of *A. nidulans* and propose that Saga/Ada plays a key role in the integration of external stimuli from interacting microbes.

Deletion of the N-acetyltransferase gene *nnaB* leads to the formation of pheofungins A–D in *A. nidulans*

To control the production of secondary metabolites at certain developmental stages or under specific environmental conditions, fungi employ a finely tuned system of global and specific regulatory mechanisms. It has been demonstrated that epigenetic regulatory processes such as histone deacetylation and DNA methylation direct the transcription of fungal genes. The deletion of a gene that encodes an *A. nidulans* histone deacetylase (HDAC) as well as the treatment of fungal cultures with HDAC inhibitors causes transcriptional activation of secondary metabolite gene clusters and production of several natural compounds. While searching for novel ways to tap the metabolic potential of fungi, we generated a number of *A. nidulans* mutant strains that lack genes that encode putative acetyltransferases, and investigated these strains with regard to changes in phenotype and secondary metabolite production. Our attention was drawn to a slowly growing strain that lacks the gene AN2745 that we renamed to *nnaB* (nidulans N-acetyltransferase B). The strain also showed a significantly increased production of secondary metabolites. These effects were clearly correlated to the absence of *nnaB*. Profiling of the Δ*nnaB* culture extract revealed besides several orsellinic acid derived phenolic compounds other previously unknown heterocyclic products, which displayed an intensely red colour. These compounds were isolated and their structures were elucidated as benzopyrano-benzothiazinones, which were termed pheofungins and had never been seen in fungi before. Interestingly, the unusual heterocyclic core is reminiscent of the well-known reddish-brown pheomelanins found in mammalian red hair (e.g. in the familiar red hair phenotype of Celtic origin) and red bird feathers. Profiling experiments indicated that pheofungin biosynthesis requires orsellinic acids and cysteine. Though their biological function remains unclear, pheofungin production is most likely triggered by intracellular stress induced by defective N-terminal protein acetylation suggesting that targeting other post-translational modifications is likely a promising route for natural product discovery.

Activation of a silent fungal polyketide biosynthesis pathway through regulatory cross talk with a cryptic nonribosomal peptide synthetase gene cluster

Combining genomic data, genetic engineering and analytical techniques is a promising avenue to discover novel natural products. A more targeted approach to activate a specific silent fungal gene cluster is the controlled expression of a pathway-specific transcription factor gene. Since various biosynthesis gene clusters contain such putative activator genes, this method is more generally applicable. Screening the model fungus *A. nidulans* we noted a silent gene cluster on chromosome II comprising two NRPS genes, *inpA* and *inpB*, flanked by a regulatory gene that we named *scpR* (for secondary metabolism cross pathway regulator). The induced expression of *scpR* using the promoter of the alcohol dehydrogenase AlcA led to the transcriptional activation of both the endogenous *scpR* gene

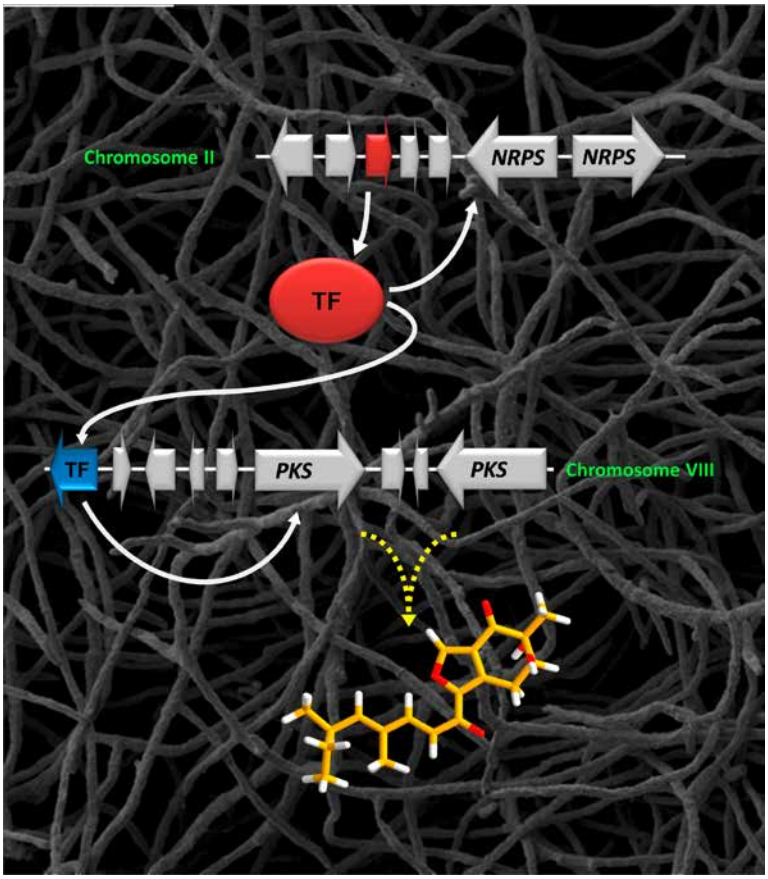


Figure 7
Model of the regulatory cross talk between two biosynthesis gene clusters located on different chromosomes of *A. nidulans*.

and the NRPS genes. Surprisingly, metabolic profiling of the *scpR* overexpressing strain revealed the polyketide asperfuranone. Indeed, we found the asperfuranone biosynthesis gene cluster located on chromosome VIII specifically induced. qRT-PCR proved the transcription of the corresponding PKS genes *afoE* and *afoG*, but also of their activator *afoA* under *alcAp-scpR* inducing conditions. By deleting the NRPS gene *inpB* and, in addition, overexpressing *scpR* we could exclude that the product of the *inp* cluster induces asperfuranone biosynthesis. Moreover, the existence of the polyketide product asperfuranone indicated that the transcription factor ScpR controls the expression of the asperfuranone biosynthesis gene cluster (Figure 7). This expression as well as the biosynthesis of asperfuranone were abolished after deleting the asperfuranone activator gene *afoA*, indicating that ScpR binds to the *afoA* promoter. This is the first report on a regulatory cross talk between two biosynthesis gene clusters located on different chromosomes.

5 Jena Microbial Resource Collection

Group Leaders: Kerstin Voigt, Axel A. Brakhage

De novo sequenced genome from *Lichtheimia corymbifera*, an ancient human pathogenic basal lineage fungus causing mucormycoses

Mucoralean fungi are able to cause severe infections in animals and humans. Although these mucormycoses are regarded as uncommon infections the number of cases increased during the last decades. The majority of mucormycotic infections is caused by *Rhizopus*, *Mucor* or *Lichtheimia* species, comprising 70 to 80% of all clinical cases of zygomycoses. While the genera *Rhizopus* and *Mucor* are closely related and derived mucoralean fungi,

Lichtheimia represents a more basal group of mucoralean fungi which also includes other pathogenic species like *Rhizomucor pusillus*. In addition, the three genera show differences in growth kinetics and morphology (e.g. dimorphism), thermotolerance and resistance to antifungals.

The genomes of *Rhizopus oryzae* and *Mucor circinelloides* were already sequenced, whereas no genomic data of more basal mucoralean pathogens are available. Here we present first insights into the genome of *L. corymbifera*. Based on 454 and Illumina sequencing data contigs with a total length of 33.6 MB were assembled. Gene prediction using AUGUSTUS resulted in 11,614 predicted genes with an average transcript length of 1308 bp. Functional annotation, investigation of alternative splicing and gene cluster analyses will help to improve the understanding of pathogenesis and pathogenicity mechanisms of zygomycotic infections. Moreover, the genome will contribute to understand the evolution of genes and gene clusters in the fungal kingdom.

Lichtheimia corymbifera is the most ancient mucoralean human pathogenic fungus which has been genome sequenced so far. The genome of *Lichtheimia corymbifera* is haploid. Codon usage deviates from aspergilli and *Candida*, both model systems commonly used to study pathogenesis in humans. Large extensions of gene expansions (gene duplication) in the *Lichtheimia* genome were detected, which are unique in the fungi genomes sequenced up to date. The number of transcription factors, phosphatases and putative proteins involved in the MAPK and calcineurin signalling pathways and iron-uptake (e.g. high affinity iron permease gene [FTR1] required for iron transport in iron-depleted environments) pathways are multiplied (doubled, triplicated). Some duplications are unique for *Lichtheimia* and *Aspergillus*, both exhibiting similar etiologies in the human patients. Genomic islands were detected where horizontal gene transfer may have occurred. The affected genes do not follow a species phylogeny.

De novo sequenced genome from _Conidiobolus coronatus_, an ancient human pathogenic basal lineage fungus causing entomophthoromycoses

Zygomycetes, formerly described as class within the fungal kingdom, are polyphyletic, and therefore, split into five distinct subphyla, which are the Entomophthoromycotina, Mucoromycotina, Mortierellomycotina, Kickxellomycotina and Zoopagomycotina. The former two subphyla contain species which are human pathogenic causing infections with diverse predisposition and etiologies. They encompass ubiquitously distributed insect-killing, saprotrophic soil- or dead plant material-inhabiting fungi of the orders Entomophthorales and Mucorales, respectively. Human pathogenic species inhabit different growth temperature optima ranging from 33 °C to 42 °C, only members of these two orders are capable of causing diseases, entomophthoromycosis and mucormycosis, in immunocompromised and immunocompetent humans, respectively. One new genome project was initiated on *Conidiobolus coronatus* and the results are discussed with respect to the evolution of genes and gene clusters involved in the development of pathogenicity. Based on Illumina sequencing data contigs with a total length of ca. 40 MB were assembled using four different assembler tools. Gene prediction using AUGUSTUS and GeneID resulted in over 10,000 predicted genes with an average transcript length of about 1,300 bp. Functional annotation, gene cluster and phylome analyses were applied to improve the understanding of gene involvement in pathogenesis of zygomycotic infections in comparative analyses. Also the data are embedded in phylome and phylogenomic studies comprising all publicly available fungal genomes and EST databases and analysed with state of the art bioinformatic approaches. Moreover, the novel genome project is discussed with respect to the 1000 Fungal Genome Project which has been newly launched last year (http://1000.fungalgenomes.org).

Conidiobolus coronatus represents the most ancient human pathogenic fungus which

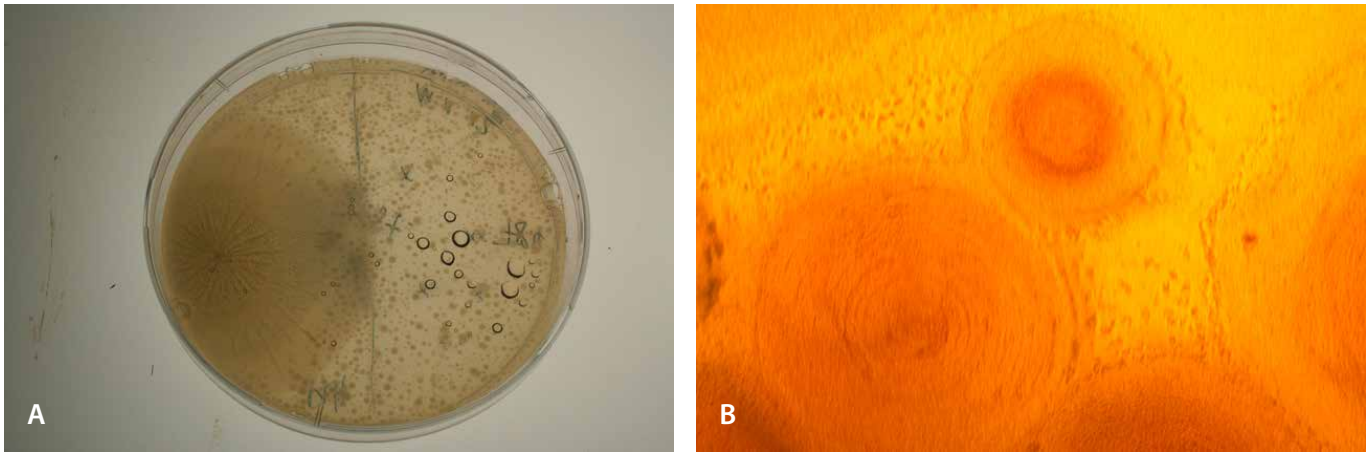


Figure 8
Isolation of endobacteria from *Condiobolus coronatus*. **(A)** macromorphology of bacterial colonies, **(B)** micromorphology of bacterial colonies.

has been genome sequenced. The genome of *Conidiobolus coronatus* contains a separate and complete bacterial genome resembling in the family Moraxellaceae (99 % match with *Enhydrobacter aerosaccus* based on the 16S rDNA). The ability of the fungus to perform hemolysis (in both insect hemocytes and sheep erythrocytes) and keratine degradation is unique in this combination. Hemolysis was detected to be highly probable of endobacterial origin. Curation of the endobacteria did not succeed so far. Genome architecture is highly unique by large extensions of gene expansions and putative horizonal gene transfer events between fungus and host insects.

Exploring the pathogenic potential of *Lichtheimia* spp.

Although the number of mucormycosis cases has increased during the last decades, little is known about the pathogenic potential of most mucoralean fungi. Among the most common agent responsible for mucormycosis are species of the genus *Lichtheimia*. *Lichtheimia* species represent the second and third most common cause of mucormycosis in Europe and worldwide, respectively. To date only three of the five species of the genus have been found to be involved in mucormycosis, namely *L. corymbifera*, *L. ramosa* and *L. ornata.* However, it is not clear whether the clinical situation reflects differences in virulence between the species of *Lichtheimia* or whether other factors are responsible. In this study the virulence of 46 strains of all five species of *Lichtheimia* was investigated in the chicken embryo model. Additionally, strains of the closest-related genus *Dichotomocladium* were included to determine the distribution of virulence within the family Lichtheimiaceae. Full virulence was restricted to the clinically relevant species while all strains of *L. hyalospora*, *L. sphaerocystis* and *Dichotomocladium* species were found to be attenuated. Although virulence differences

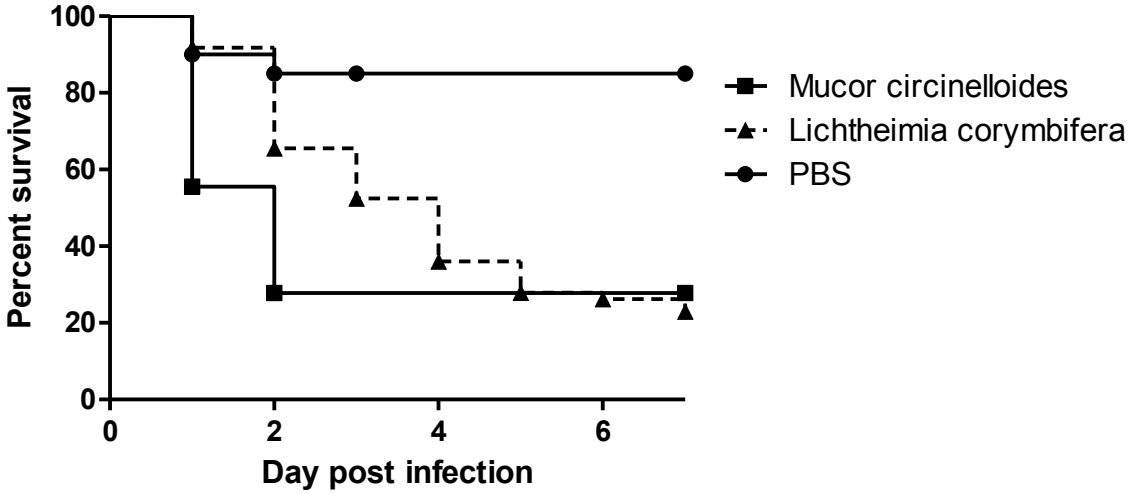


Figure 9
Virulence of *Lichtheimia corymbifera* and *Mucor circinelloides* as evaluated in the embryonated hen egg model.

were present in the clinically relevant species, no connection between origin (environmental *vs.* clinical) or phylogenetic position within the species was observed. Combined with additional data about e.g. stress resistance, nutritional requirements or optimal growth temperatures, the current results suggest that the clinical situation in fact reflects the different virulence potential of the Lichtheimiaceae. Slower growth at 37°C might explain low virulence of *L. hyalospora*, *L. sphaerocystis* and *Dichotomocladium*; however, similarly slow growing strains of *L. ornata* were fully virulent. Thus, additional factors or a complex interplay of factors determines the virulence of strains. Our data suggest that the clinical situation in fact reflects different virulence potential of Lichtheimiaceae.

An embryonated hen egg model was established for *Lichtheimia* which allows a high throughput test for the virulence potential. Clinically relevant species correlated with high virulence whereas non-clinically relevant species do not correlate. The ability to grow above 37°C does not correlate with virulence, thus thermotolerance is an essential prerequisite of virulence but not the exclusive one.

Mycoparasitism of mucoralean fungi (Mucoromycotina, "Zygomycota")

Fungi, like other microorganism, interact with the environment and other organisms. Besides the morphological variability of fungi also the lifestyle differs among species (saprotrophism, symbiosis, parasitism). Many fungi are known to be parasites of plant and animals but there is also parasitism between fungi ("mycoparasitism"). Depending on the damage of the host mycoparasitism can be classified into necrotrophic and biotrophic parasitism. Mycoparasitism in "Zygomycetes" can occur between species inside and outside of the subphylum. The topic of this

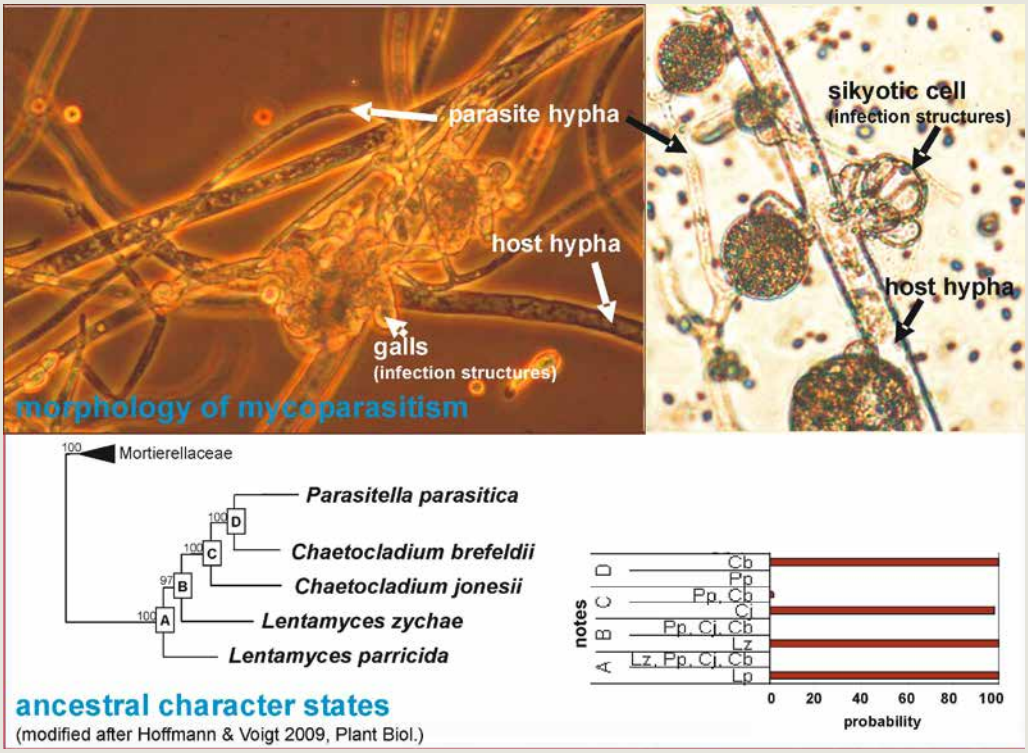


Figure 10
Morphology and ancestral character state analysis shows the evolution of mycoparasitism starting from *Lentamyces parricida* as the most ancient mycoparasite among the Mucorales.

project is the biotrophic fusion parasitism, a special form of mycoparasitism, which occurs only between mucoralean fungi. Only three genera and four species are known to be mycoparasites namely *Lentamyces parricida*, *Parasitella parasitica*, *Chaetocladium brefeldii* and *Chaetocladium jonesii*. Like other parasites all species are able to grow saprotrophic independent of the host.

Transcriptomic analyses based on a pooled cDNA population derived from expression of genes under 100 physiological conditions revealed over 10,000 genes, which represent almost the whole gene set of *Lentamyces*, which is the second ancient genus of the order Mucorales. Evidences were obtained for a link between mycoparasitism and pathogenesis in humans regarding host recognition *via* specific surface receptors (CotA). Moreover, physiological and genetic analysis of the most basal mycoparasite *Lentamyces parricida* and different host species are used to clarify the evolutionary origin of mycoparasitism.

Spatial investigation of oil vesicles in Mortierellales with micro-Raman spectroscopy with respect to the evolution of the Mortierellomycotina as rapidly evolving group

The order Mortierellales comprise over 80 described species and are ubiquitous soil living "Zygomycetes". Little is known about their evolution, their global distribution, their ecological impact in the soil communities and their potential role to become human pathogens. For example, *Mortierella wolfii* is able to grow at 37°C and causes abortion in cattle and swine with serious damages in agriculture. From a biotechnological point of view another species came to fame: *Mortierella alpina*.

Some strains of *M. alpina* have proven to produce high amounts of oil vesicles rich

in poly-unsaturated fatty acids (PUFA) and present a promising source for industrial exploitation. The species is also used in the production of dietary supplements and has therefore implications in white biotechnology. Furthermore, the species became a model of fatty acid metabolism. We investigated single oil vesicles inside intact hyphae using micro-Raman spectroscopy and studied the oil-producing potential of the other mortierellean species. PUFAs were found in varying amounts and quality in each of the representatives from all monophyletic groups. However, little is known about how or why the fungi produce these oil vesicles in the first place. Knowledge about the spatial distribution and composition could help to understand the role of the oil for the fungus. For spatial investigation hyphae of various *Mortierella* species were successfully grown onto microscopic slides. Multiple oil vesicles in single hyphae of various lengths (up to several mm) were investigated spectroscopically. From the spectra, information about the total degree of unsaturation was extracted. Comparison of the oil spectra from different vesicles within one hypha with the ones from different hyphae of one sample shows the same degree of unsaturation within one sample irrespective of the measurement site. The degree of unsaturation of the fungal oils does neither change over the length of a hypha irrespective of morphological changes nor within one sample. No preference for the accumulation of the oils at specific sites of the hypha could be identified.

A complete revision of the systematics based on a comprehensive species phylogeny was obtained. The Mortierellales are a rapidly evolving group. Lipid composition and PUFA profiles correlate with a species phylogeny. The fatty acids produced by Mortierella have large potential as nutrition additives as sea fish supplements.

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Deutsche Forschungsgemeinschaft
Jena School for Microbial Communication
Project: *Aspergilllus fumigatus* – Analysis of the interaction of the human pathogenic fungus *Aspergilllus fumigatus* with immune effector cells by functional genomics
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Deutsche Forschungsgemeinschaft
Jena School for Microbial Communication
Project: Redox regulation, development and hyphal growth in *Aspergillus nidulans*
Axel Brakhage, Peter Hortschansky

Deutsche Forschungsgemeinschaft
SPP1160 Human-pathogenic Fungi
Project: Identification of virulence determinants of the human-pathogenic fungus *Aspergillus fumigatus* by proteome analysis
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Deutsche Forschungsgemeinschaft
SPP1160 Human-pathogenic Fungi
Project: 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*
Reinhard Guthke, Axel Brakhage

Deutsche Forschungsgemeinschaft
SPP1160 Human-pathogenic Fungi
Coordination
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European Union
Framework Programme 6 Marie Curie Research Training Networks (RTN)
SIGNALPATH: MAP kinase cascades controlling virulence in fungi: from signals to pathogenicity response
Axel Brakhage, Thorsten Heinekamp

European Union
Framework Programme 6 Specific Targeted Research Project
MANASP: Development of novel management strategies for invasive aspergillosis
Axel Brakhage, Olaf Kniemeyer

European Union
Framework Programme 7 European Re-integration Grants (ERG)
CellWallPath – Identification of new molecules able to bypass the cell wall compensatory pathways in the pathogenic fungus *Aspergillus fumigatus*
Vito Valiante

European Union
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ARIADNE – Signaling circuitry controlling fungal virulence: identification and characterization of conserved and specific fungal virulence genes as common antifungal targets
Axel Brakhage, Thorsten Heinekamp

Freistaat Thüringen
ProExzellenz Initiative
NanoConSens – Nanocontainer und nanostrukturierte Trägermaterialien für Sensorik und Wirkstofftransport
Axel Brakhage, Christian Hertweck

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ProExzellenz Initiative
MikroPlex – Analyse mikrobieller Kontaminationen und ihrer Sekundärmetabolite in komplexen Medien
Axel Brakhage, Volker Schroeckh

German Israeli Foundation
The hypoxic response in the pathogenic mold *Aspergillus fumigatus* and its relevance to disease
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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 *in vivo* biopolymer-interaction technologies and multicolour 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 genomics, 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, i.e. it combines the high spatial resolution and anatomical detail of CT with the molecular, quantifiable images obtained by PET. Comparative genomics, 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

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, wirts-spezifischen Antworten wirkungsvoll erfassen zu können, entwickeln wir hochmoderne Mikro- und Nanosysteme. 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 *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 Genomics, 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 Genom-, 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 Weise aufeinander treffen. Außerdem liefern diese Systeme, kombiniert mit

Campus in Jena, where research interests in different fields, such as physics, chemistry, and biology meet. Combined with the automation of techniques, these systems will also provide effective tools for the rapid realisation 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 theoretically and practically with modern aspects of basic and applied research.

During the last two years, several bachelor, master, 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 thermo cycling 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 hyper spectral imager coupled with multivariate data analysis provides a powerful new tool for understanding complex biological and biomedical samples.

der Möglichkeit zur Automatisierung, eine Voraussetzung für die effiziente Realisierung von Produkten und Instrumenten, was für die „BioRegio" Jena ein nicht unwesentlicher Aspekt ist.

Um eine optimale Erweiterung durch neueste wissenschaftliche Erkenntnisse und technische Mittel zu haben, kooperieren wir mit mehreren lokalen und internationalen Instituten und Industrieunternehmen. Die Erfahrungen und Kenntnisse, die wir aus unseren Arbeiten gewinnen, erlauben es uns, Studenten theoretisch und praktisch mit modernsten Aspekten der Grundlagenforschung und angewandten Wissenschaft zu konfrontieren. So betreuten wir in den letzten zwei Jahren wiederum mehrere Bachelor- und Masterstudenten, Diplomanden und Doktoranden in unserer Abteilung, am Leibniz-Institut für Altersforschung – Fritz-Lipmann-Institut (FLI) und am Max-Planck-Institut für chemische Ökologie, 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. Anderseits 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 Phagocytosis of melanised *Aspergillus* conidia by macrophages exerts cytoprotective effects by sustained PI3K/Akt signalling

Group Leaders: Hans Peter Saluz (in cooperation with Axel A. Brakhage, Dept Molecular and Applied Microbiology)

Host cell death is a critical component of innate immunity and often determines the progression and outcome of infections. The opportunistic human pathogen *Aspergillus fumigatus* can manipulate the immune system either by inducing or inhibiting host cell apoptosis dependent on its distinct morphological form. Here, we show that conidia of *Aspergillus* sp. inhibit apoptosis of macrophages induced via the intrinsic (staurosporine) and extrinsic (Fas ligand) pathway. Hence, mitochondrial cytochrome c release and caspase activation were prevented. We further found that the anti-apoptotic effect depends on both host cell *de novo* protein synthesis and phagocytosis of conidia by macrophages. Moreover, sustained PI3K/Akt signalling in infected cells is an important determinant to resist apoptosis. We demonstrate that pigmentless *pksP* mutant conidia of *A. fumigatus* failed to trigger protection against apoptosis and provide evidence that the sustained survival of infected macrophages depends on the presence of the gray-green conidial pigment consisting of dihydroxynaphthalene-melanin. In conclusion, we revealed a novel potential function of melanin in the pathogenesis of *A. fumigatus*. For the first time, we show that melanin itself is a crucial component to inhibit macrophage apoptosis which may contribute to dissemination of the fungus.

Programmed cell death (apoptosis) is a well known mechanism involved in the regulation of innate immunity. The manipulation of host cell apoptosis is a critical determinant in the progression and outcome of an infection mediated by a variety of pathogens. In principal, two regulatory mechanisms have been described: induction and inhibition of host cell apoptosis. As an example, *Candida albicans* induces apoptosis to evade phagocytic killing. Therefore, the fungus can penetrate the epithelial barrier to gain access to the submucosa. On the other hand, suppression of the host apoptotic response is a mechanism used by *Leishmania sp.* As a result, the pathogen can survive in an intracellular niche that also facilitates its spreading. For the human pathogenic fungus *Aspergillus fumigatus*, the most common cause of invasive aspergillosis (IA) in patients with severely impaired immunity, both regulatory mechanisms have been described, depending on its morphological form. Conidia of *A. fumigatus* were shown to inhibit apoptosis induced by different stimuli in macrophages, epithelial cells, and pneumocytes. Hyphae, on the other hand, secrete the secondary metabolite gliotoxin, which induces apoptosis in host cells thereby suppressing immune responses. We and others previously showed that *A. fumigatus* conidia inhibit host cell apoptosis in a caspase-3 dependent manner (Volling *et al.*, 2011 and references therein). However, the detailed mechanism of apoptosis inhibition is still a matter of debate. Apoptosis can be initiated via two major signalling pathways: the extrinsic death receptor pathway and the intrinsic mitochondrial cytochrome c/Apaf-1 pathway. Stimulation of death receptors such as CD95 (Fas) leads to the activation of the extrinsic pathway mediated by the initiator caspase-8. By contrast, intracellular pro-apoptotic signals, such as formation of reactive oxygen species (ROS) or chemical agents, induce the intrinsic pathway leading to mitochondrial outer membrane permeabilization with subsequent release of pro-apoptotic intermembrane space

proteins, e.g., cytochrome c, into the cytosol. As a result, the initiator caspase-9 is activated. Both pathways converge on the activation of executioner caspases (caspase-3, -6, and -7), which are responsible for the biochemical and morphological changes associated with apoptosis. The apoptotic pathway is strictly regulated by inhibitor of apoptosis proteins (IAPs) that directly bind and consequently prevent effector caspase activation and pro- and anti-apoptotic members of the Bcl-2 protein family which control mitochondrial outer membrane permeabilization and cytochrome c release. Furthermore, several host cell signalling pathways are involved in apoptosis regulation. One of them is the phosphatidylinositol-3-kinase (PI3K)/Akt signalling pathway. In addition to playing an important role in mediating survival of monocytes/macrophages in response to growth factors and cytokines and blocking apoptosis by toxic stimuli, the PI3K/Akt pathway was shown to be implicated in the anti-apoptotic property of various pathogens. Stimulation of the PI3K/Akt pathway via the downstream serine/threonine kinase Akt (also referred to as protein kinase B (PKB)) affects the balance of anti- and pro-apoptotic Bcl-2 family members by, for example, regulating the expression or protein stabilisation of anti-apoptotic Bcl-2 and Mcl-1 or by direct phosphorylation of pro-apoptotic members, e. g., Bad and Bax, thus inhibiting their activity. Akt also phosphorylates Forkhead box class O (FoxO) transcription factors and activates the inhibitor of NF-KB (IKB) kinase (IKK), which results in phosphorylation-dependent inactivation of I·B and subsequent release and translocation of NF·B to the nucleus where this factor induces the transcription of proinflammatory and survival related genes. As melanin is implicated in the virulence of *A. fumigatus*, the aim of the study was to examine the role of the conidial pigment dihydroxynaphthalene (DHN)-melanin in sustained survival of infected macrophages. First we showed that *A. fumigatus* conidia inhibit apoptosis of murine alveolar macrophages, the prominent resident phagocytic cells in the alveolar tract, in a caspase-3 dependent mechanism. Then, we demonstrated that this phenotype extends to human primary monocyte-derived macrophages and a murine monocyte-macrophage cell line. The protective capacity of conidia on macrophage apoptosis was found to include both extrinsic and intrinsic apoptotic stimuli. This conclusion was based on the observation that apoptosis induced by both the kinase inhibitor STS and Fas ligand was inhibited by wild-type conidia. In particular, the release of mitochondrial cytochrome c into the cytosol and the activation of the initiator caspase-8 and -9 as well as executioner caspase-3, -6, and -7 were reduced in infected macrophages. Whereas apoptosis induced by STS is considered as artificially-induced cell death, Fas ligand induced apoptosis of infected and/or non-infected bystander cells such as lymphocytes, macrophages, and neutrophils was shown to be implicated in the pathogenesis of various viral, bacterial, mycobacterial, and parasitic infections. Consistently, it was demonstrated that the death receptor Fas is down-regulated in human monocytes upon *A. fumigatus* infection which would render infected cells resistant against Fas ligand-induced apoptosis. Because heat-killed conidia are still able to prevent macrophage apoptosis and a spore diffusate did not render macrophages resistant against chemically induced apoptosis, we concluded that a surface factor present on conidia might be responsible for the anti-apoptotic effect rather than an actively secreted product. Resting conidia are covered by a proteinaceous hydrophobic rodlet layer comprised of RodA protein that is released during swelling. This layer was shown to render conidia immunologically inert and reduce formation of neutrophil extracellular traps. In addition, DHN-melanin is present in the outermost cell wall of *A. fumigatus* conidia. By analysing the effect of *A. fumigatus* melanin mutant strains, melanin ghosts, and isolated DOPA-melanin in addition to a strain lacking the rodlet layer on apoptosis in macrophages, we identified melanin, but not the rodlet layer, as a decisive determinant for the anti-apoptotic property of *A. fumigatus* co-

nidia. This finding well agress with the notion that non-melanised white conidia, as in the case of the *pksP* mutant, exhibited reduced virulence in a murine model of invasive aspergillosis. It was shown that DHN-melanin led to reduction of phagocytosis and maturation of phagolysosomes. Furthermore, the pigment plays a protective role against ROS 1 produced by immune cells. Inhibition of host cell apoptosis is presumably another regulatory mechanism mediated by DHN-melanin to interfere with the innate immune response. The observation that Δ*arp*2 and Δ*abr*2 conidia, which produce DHN-melanin precursors, inhibited apoptosis similarly to wild-type conidia and that these strains were virulent in the mouse model of invasive aspergillosis strongly supports the relevance of apoptosis inhibition for *A. fumigatus* pathogenesis. Since different types of melanin (DHN-melanin present in *A. fumigatus* conidia and melanin ghosts, isolated DOPA9 melanin, and melanin present in *A. nidulans* and *A. niger*) had the same inhibitory impact on macrophage apoptosis, the anti-apoptotic property seems to be attributed to a general characteristic of melanin molecules. Due to the presence of stabilised free radicals, melanin can act as a trap for unpaired electrons arising from ROS and neutralise antifungal drugs, such as amphotericin B, still used for treatment of invasive fungal infections. Treatment of cells with STS and Fas ligand triggers mitochondrial production of ROS, and generation of ROS within mitochondria and lysosomes has been reported to promote apoptotic cell death. Hence, it is possible that melanin on the surface of *A. fumigatus* conidia inhibits apoptosis by quenching ROS produced in response to apoptosis induction. Similarly, it was shown that *Sepia* melanin (DOPA-melanin) protects retinal pigment epithelium against blue light-induced apoptosis. It has been suggested that this effect is due to efficient light absorption and ROS quenching through melanin free radicals. Inhibition of apoptosis may be a general mechanism of immune evasion that *A. fumigatus* employs during infection, as similar findings have been reported for several cell types. Pneumocytes and bronchial epithelial cells exposed to *A. fumigatus* conidia were protected against apoptosis upon TNF-α and STS treatment. These studies support our observations but also suggest that the anti-apoptotic mechanism applied by *A. fumigatus* conidia may vary in different cell types. For example, in epithelial cells, an until now unknown anti-apoptotic factor released by *A. fumigatus* conidia is responsible for the sustained survival of infected cells and killed conidia no longer inhibit apoptosis. By contrast, we did not observe an inhibitory effect of a conidial diffusate on macrophage apoptosis. Moreover, our data strongly suggest that phagocytosis of melanised *A. fumigatus* conidia and inhibition of phagolysosomal acidification is required to prolong survival of infected macrophages instead. It is interesting to note that conidia of *A. nidulans* and *A. niger* inhibited apoptosis in macrophages in a way similar to *A. fumigatus* wild-type conidia. Conversely, conidia of these strains had no inhibitory effect on apoptosis in bronchial epithelial cells. The discrepancy between these observations may be explained by the fact that the anti-apoptotic mechanism in macrophages depends on the presence of melanin, which is synthesised by all *Aspergillus* sp. albeit the type of melanin varies, whereas apoptosis of epithelial cells was suggested to be inhibited by an actively secreted factor of the fungus.

In *A. fumigatus*, a reduced acidification of phagolysosomes is dependent on a functional *pksP* gene. By using the chemical inducer of phagolysosome acidification chloroquine, we demonstrated that wild-type conidia exhibited an anti-apoptotic effect only when residing in phagolysosomal compartments with a neutral pH. Interactions of melanin free radicals with small molecules, e.g., metal ions and drugs, are the most effective at a pH between 6 and 8. Thus, it is tempting to speculate that acidification of phagolysosomes might change the anti-apoptotic property of melanin present on conidia by for example affecting the interaction with proteins

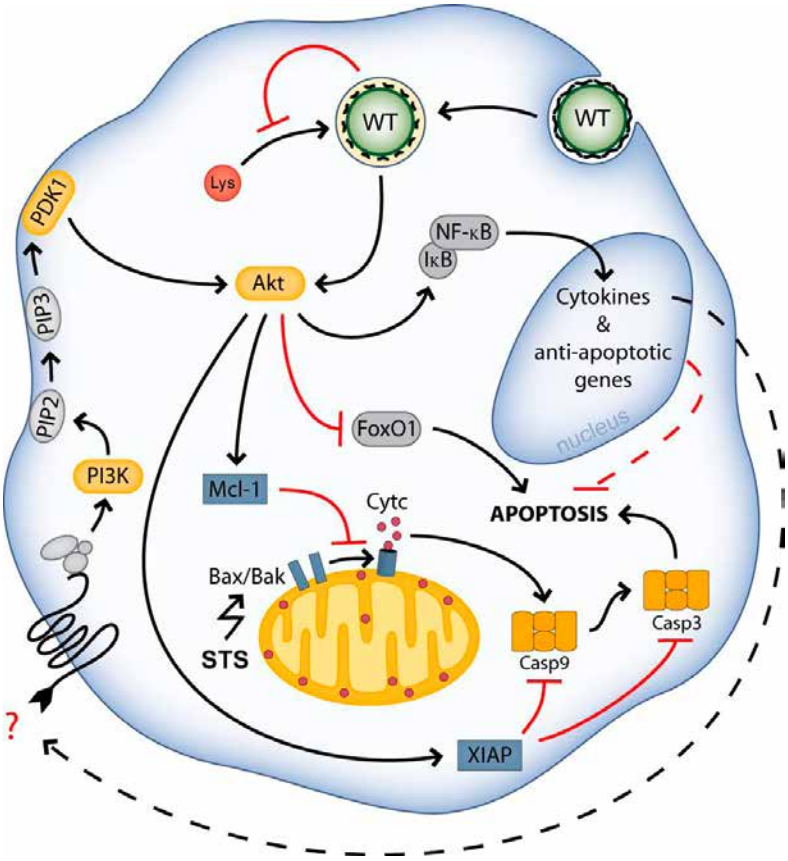

Figure 1
Model of apoptosis inhibition of *A. fumigatus* conidia in macrophages.

or small molecules. Various pathogens such as *Chlamydia trachomatis* and *Leishmania major* engage the PI3K/Akt signalling pathway in order to sustain survival of infected cells in response to stress and death receptor signals. Here, we revealed that activation of the PI3K/Akt pathway is also a mechanism used by *A. fumigatus* to prolong the survival of infected macrophages. This finding contradicts data of Bonifazi *et al.* (2010) who demonstrated that only *A. fumigatus* hyphae and swollen conidia, but not resting conidia, activate PI3K/Akt signalling in a dectin-1 and TLR2 dependent manner in DCs. Nonetheless, previously it also was shown that phagocytosis of *A. fumigatus* resting conidia by macrophages depends on PI3K signalling. Since we analysed phosphorylation of Akt 6 h after co-incubation of macrophages with *A. fumigatus* conidia, it is likely that meanwhile conidia had swollen within the phagolysosomes. Such conidia are recognised by the host cell and thus induce PI3K/Akt signalling. Recently, the importance of the PI3K/Akt signalling pathway for *A. fumigatus* pathogenesis was demonstrated *in vivo*. Intranasally delivered small interfering RNA (siRNA) or chemical inhibitors of the PI3K pathway modified the inflammatory response and impaired resistance to infection. Because PI3K/Akt signalling controls various cellular responses including inflammatory cytokine production and cell survival, it is conceivable that PI3K inhibition controls cytokine production via modulation of the survival of infected macrophages.

The serine/threonine kinase Akt is believed to modulate the activity of several pro- and anti-apoptotic molecules of the Bcl-2 family, which control mitochondrial homeostasis and release of cytochrome c. These molecules include the anti-apoptotic members Bcl-2 and Mcl-1 in addition to the pro-apoptotic members Bad and Bax. Furthermore, Akt regulates the activity of transcription factors of

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the Forkhead box, class O family (FoxO) and IKK. Here, we identified the Akt downstream targets Mcl-1, FoxO1, and IKK as being differentially regulated in wild-type compared to *pksP* mutant infected cells. Moreover, we demonstrated that this difference is due to the presence of melanin. PI3K/1 Akt signalling has been shown to control the survival of *Chlamydia trachomatis* infected cells via Mcl-1 expression. Since Mcl-1 plays an important role in maintaining mitochondrial homeostasis, a sustained level of Mcl-1 in *A. fumigatus*-infected cells upon apoptosis induction could account for the reduced level of cytosolic cytochrome c and inhibition of caspase processing. A possible role of Mcl-1 in protection of *A. fumigatus*-infected cells from apoptosis is supported by our observation that CHX-induced translation inhibition compensated for the anti-apoptotic effect of wild-type conidia. Previously, it was demonstrated that in response to protein synthesis inhibition, pro-apoptotic Bik binds to and antagonises Mcl-1 to activate Bak, leading to mitochondrial cytochrome c release and apoptosis.

In conclusion, for the first time, our results demonstrate that melanin itself is a crucial component to prevent macrophage apoptosis by a mechanism that involves PI3K/Akt signalling. Our data suggest that *A. fumigatus* uses this mechanism to reside in an intracellular niche that prolongs conidial survival within the host.

2 Protein-protein interaction networks during infection and cellular stress

Group Leader: Frank Hänel (in cooperation with Frank Grosse and Karl Otto Greulich, Leibniz Institute for Age Research – Fritz Lipmann Institute, Jena, Germany)

Interactions of type III-secreted chlamydial proteins with host proteins

The avian and human pathogen *Chlamydia psittaci* (formerly *Chlamydophila psittaci*) is the causative agent of psittacosis and represents the most important animal chlamydiosis of zoonotic character. All members of the family *Chlamydiaceae* are obligate intracellular parasites that develop in a host cell within an inclusion, i.e. a membrane-bound compartment that does not fuse with lysosomes. The membrane of the inclusion is initially formed by 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 non-infectious but metabolically active reticulate bodies (RB), which proliferate within the expanding inclusion, giving rise to 1000 or more progeny per host cell. The developmental cycle ends after 2-3 days depending on the strain, when RBs transform back into EBs and are released into the extracellular medium. During this unique biphasic developmental cycle replicating bacteria acquire energy and biosynthetic precursors from the infected cell. Furthermore, chlamydiae modulate cellular functions such as apoptotic programme and immune response (Cocchiaro and Valdivia, 2009). Studies on inhibitors of bacterial protein synthesis suggest that modulation of the host cell functions requires the activity of chlamydial proteins. All *Chlamydiaceae* possess genes encoding 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 chlamydial effector proteins are targeted by the TTS to the inclusion membrane. The first set of chlamydial effector proteins identified was a family of integral inclusion membrane (Inc) proteins that share one remarkable feature, i.e. they possess a very large (50-80 amino acids) bilobed hydrophobic domain, a secondary structure motif predictive of protein localisation to the chlamydial inclusion membrane. The first member of the family of Inc proteins identified, IncA, is the one that has attracted most of the attention. First cloned in *C. caviae* it has known homologs in *Chlamydia trachomatis*, *C. pneumoniae*, *C. felis*, *C. muridarum*, *C. abortus* and *C. psittaci.* The level of sequence similar-

ity among the homologs is low, and antibodies against IncA do not cross-react with other chlamydial species. Furthermore, in all IncA proteins identified so far, SNARE (soluble N-ethylmaleimide-sensitive factor attachment protein receptor)-like motifs were identified. These motifs allow interactions with several host SNARE proteins, which are essential for membrane fusion. In addition to the bilobed hydrophobic domain Inc proteins, such as IncA and IncG, harbour domains exposed to the cytoplasmic side of chlamydial inclusion where they mediate interactions with eukaryotic host proteins. Thus, Inc proteins are probably central regulators of pathogen-host interactions (Dehoux *et al.*, 2011).

Using the yeast two-hybrid technology, we utilised IncA of *Chlamydia psittaci* as bait in a spate of interaction screens against a human HeLa cDNA library pretransformed into yeast. In this way we identified several host proteins specifically interacting with IncA in the yeast system. Selected protein interaction pairs were the subjects of continuative biochemical, physiological and cell biological investigations. One example of a more in-depth characterised protein-protein interaction is the interplay of IncA with host protein Ras-GTPase activating protein SH3 domain binding protein 1 (G3BP1). G3BP1 was initially identified as a ubiquitously expressed cytosolic 68 kDa protein that co-immunoprecipitates with Ras-GTPase-activating protein (GAP). The G3BP1 cDNA revealed that G3BP1 is a 466-amino-acid protein that shares several features with heterogeneous nuclear RNA-binding proteins, including RNA recognition motifs (RRM) RNP1 and RNP2, an RG-rich domain and acidic sequences. G3BP1 colocalises and physically interacts with GAP at the plasma membrane of serum-stimulated but not quiescent Chinese hamster lung fibroblasts. In quiescent cells, G3BP1 was hyperphosphorylated on serine residues and harbours a phosphorylation-dependent RNase activity which specifically cleaves the 3´-untranslated region of human *c-myc* mRNA. In addition to its role in Ras-GAP signalling and its function as a phosphorylation-dependent RNase several other putative biological activities of G3BP1 were suggested, i.e. involvement in NFkB and IkB nucleo-cytoplasmic equilibrium, interactions with ubiquitin-specific proteases and participation in stress-granule formation.

While there is a growing list of publications dealing with interactions between Incs from *C. trachomatis* and *C. pneumoniae*, this was, to our knowledge, the first example of a documented interaction between an Inc protein from a zoonotic chlamydia and a host protein. In GST-pull down and co-immunoprecipitation experiments, both *in vitro* and *in vivo* interaction between full-length IncA and G3BP1 could be shown. Using fluorescence microscopy the localisation of G3BP1 near the inclusion membrane of *C. psittaci*-infected Hep-2 cells was demonstrated (Figure 2). Finally, infection of Hep-2 cells with *C. psittaci* and overexpression of IncA in HEK293 cells led to a decrease in c-Myc protein concentration, but not at mRNA level. This effect could be ascribed to the interaction between IncA and G3BP1 since overexpression of an IncA mutant construct disabled to interact with G3BP1 did not cause a decrease in c-Myc concentration. Additionally, siRNA mediated knock-down of G3BP1 in Hep-2 cells had the same influence on c-Myc protein level as overexpression of IncA. We hypothesise that lowering the host cell c-Myc protein concentration may be part of a strategy employed by *C. psittaci* to avoid apoptosis and scale down host cell proliferation.

Interaction network of the multifunctional signal protein TopBP1

TopBP1 is a BRCT domain-rich protein that is structurally and functionally conserved throughout eukaryotic organisms. Though TopBP1 was initially identified as a DNA topoisomerase IIβ-binding protein (hence its name), its involvement in a number of cellular processes such as replication, transcription and DNA damage response was soon established. Moreover, very recent investigations of a conditional TopBP1 knockout mouse showed additional roles of the protein in cell prolifer-

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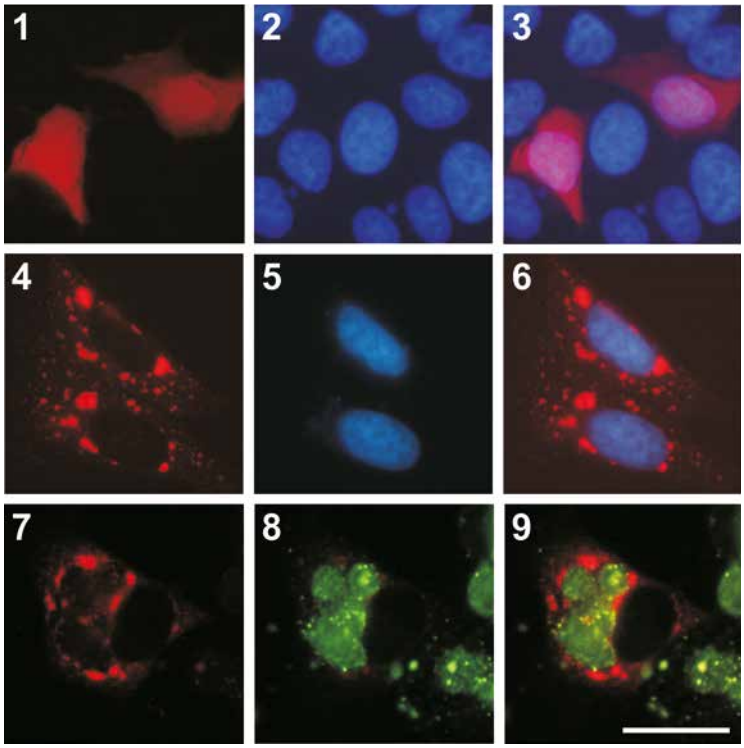

Figure 2
G3BP1 is accumulated around the chlamydial inclusion in *C. psittaci* infected Hep-2 cells. Hep-2 cells were transiently transfected with a DsRed-G3BP1 expression construct (4-6) or with DsRed vector alone as a control (1-3). Additionally, Hep-2 cells transiently expressing DsRed-G3BP1 were infected with *C. psittaci* at a MOI of 3 (7-9), and 48 h after infection this cells as well as the non-infected cells were fixed and stained with anti-chlamydial LPS (green) and DAPI (blue), and viewed under a fluorescence microscope. The DsRed (red) (1, 4, 7), DAPI (blue) (2, 5) and anti-LPS (green) (8) signals were merged (3, 6, 9). Overexpressed DsRed-G3BP1 is concentrated in stress-granules around the nucleus in uninfected cells (6). G3BP1-containing stress granules are localised around chlamydial inclusions in infected cells (9). Bar = 20 µm.

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ation and the maintenance of chromosomal integrity. The initiation of DNA replication requires the assembly of multiple protein complexes at the origins of replication. We and others have shown that TopBP1 is essential for chromatin loading of the MCM helicase activator Cdc45 at replication origins in metazoan cells (Schmidt *et al.*, 2008) and hence for the formation of the pre-initiation complex. Human TopBP1 contains nine BRCT domains that originally were identified in a number of proteins involved in DNA repair and the cell cycle checkpoint. In response to UV-caused DNA damage, several checkpoint proteins, including Rad17, the Rad9-Rad1-Hus1 complex, and the ATR/Atrip complex are recruited to RPA-coated single-stranded DNA, which leads to the subsequent phosphorylation of Chk1 by ATR (Figure 3). It was reported that TopBP1 plays a crucial role in the ATR signalling pathway by physically interacting with ATR, which dramatically enhanced the ATR kinase activity. However, the important question of how TopBP1 recognises damaged DNA remained unresolved, since TopBP1 localises to the sites of DNA damage independently of ATR. Despite the fact that BRCT regions located in the N- and C-terminal halves of TopBP1 can bind *in vitro* to DNA with bulky base lesions it is generally believed that TopBP1 interacts with proteins that sense DNA damage rather than directly participating in DNA damage detection (Garcia *et al.*, 2005). In support of this assumption we recently showed that TopBP1 interacts with PARP-1, an abundant nuclear protein involved in the DNA-base-excision-repair (Wollmann *et al.*, 2007). Another example of TopBP1 interactions with a DNA damage recognizing protein is the Nijmegen breakage syndrome protein 1. Therefore, there may exist a set of further proteins that interact with TopBP1 and mediate its recruitment to sites of DNA damage.

To further dissect the biological functions of TopBP1, we explored TopBP1-interacting

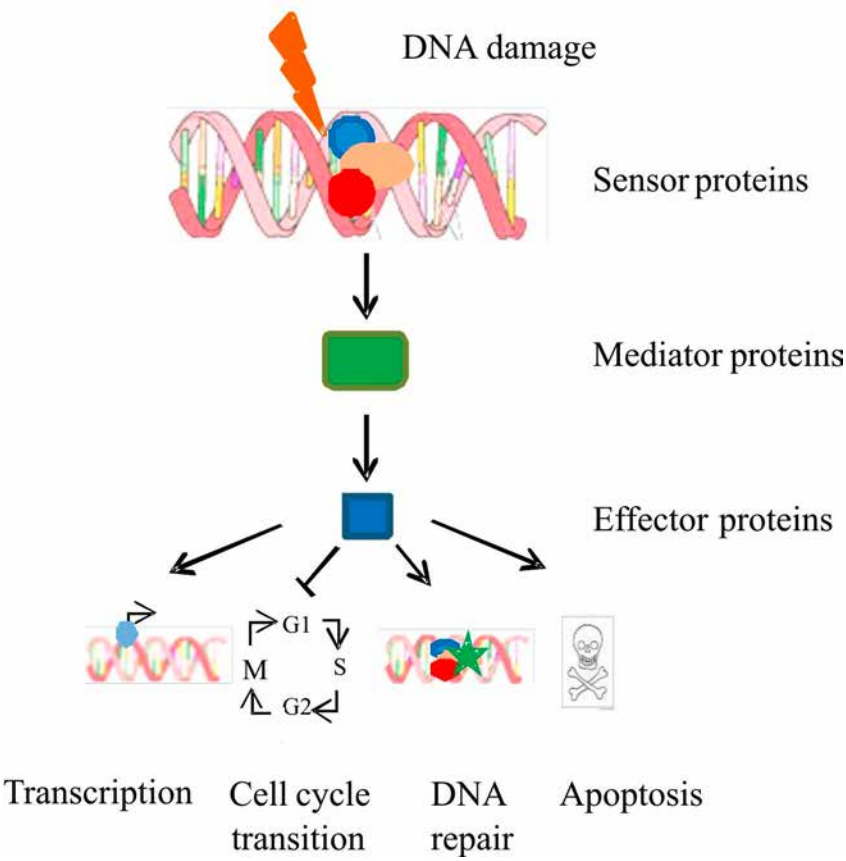

Figure 3
Conceptual organisation of the essential components and the outcomes of checkpoint responses.

proteins by co-immunoprecipitation assays and LC-ESI-MS-analyses. As TopBP1 binding partners we identified p54(nrb) and PSF, and confirmed the physical interactions by GST pull-down assays, co-immunoprecipitations and by yeast two-hybrid experiments. Recent evidence shows an involvement of p54(nrb) and PSF in DNA double-strand break repair (DSB) and radioresistance. To get a first picture of the physiological significance of the interaction of TopBP1 with p54(nrb) and PSF we investigated in real time the spatiotemporal behaviour of the three proteins after laser microirradiation of living cells. Localisation of TopBP1 at damage sites was noticed as early as 5 seconds following damage induction, whereas p54(nrb) and PSF localised there after 20 sec. Both p54(nrb) and PSF disappeared after 200 seconds while TopBP1 was retained at damage sites significantly longer suggesting different functions of the proteins during DSB recognition and repair.

3 Chlamydial population genomics

Group Leaders: Gerhard Schöfl, Hans Peter Saluz

The dramatic progress in sequencing technologies during the past couple of years allows us now to fairly easily produce large numbers of microbial genomic data sets at reasonable costs in no time. We can perform comparative analyses thus not only of distantly related genomes but also sequence and compare multiple genomes from within the same species. Consequently it has become feasible to apply the traditional tool-set and methodology of population genetics to complete genomes, which is the emerging field of 'population genomics'.

Comparative genomics, i.e. the comparison of genomes of (usually distantly related) species, and population genomics allow extracting dif-

ferent types of information about biological function and evolutionary processes from genome data. The former approach attempts to infer function from patterns of conservation across genomes, relying on the assumption that conserved amino acids and nucleotides probably perform similar functions in different organisms. Importantly, this allows us to reveal functional and evolutionary relationships for a large number of genes and their products among two or more genomes.

Population genetic theory, on the other hand, provides us with statistical tools to infer about the evolutionary forces that structure and maintain genetic variation within species. Applying population genetic methods to microbial genomes allows drawing inferences about adaptive and/or demographic processes that have shaped the genomes of closely related bacterial species since their evolutionary separation. This, in turn, may lead to the detection of genes or genomic regions that have been targets of recent selective processes. Because traces of recent selection hint at roles for such genes in coping with their ever-changing ecological niche, which for pathogenic microbes first and foremost is their host, such approaches may reveal novel factors involved in virulence or host specificity.

In 2010, we launched a project to examine genomic aspects of infection, virulence, and host-preference in the *Chlamydiacae*, a family of obligate intracellular pathogens causing a wide range of diseases in humans and animals. Being a well-sequenced group, with – at present – 17 and 5 fully sequenced genomes from the human-specific pathogens *Chlamydia trachomatis* and *C. pneumoniae*, respectively, as well as single genomes from most other members of the genus, we were seeking to apply a combination of more traditional comparative genomics and population genomic approaches.

With the two abundantly sequenced chlamydial species both showing a rather narrow host-range (that is, humans by and large), we generated the genome sequence of the type strain of a hitherto unsequenced *Chlamydia*, the avian pathogen *C. psittaci*, which exhibits a rather broad host-spectrum including birds, crocodiles, and a wide variety of mammalian hosts.

To determine its complete genomic sequence, we used a combination of Roche 454 pyrosequencing, Illumina, and Sanger methodologies, which generated approximately 28 million sequence reads that could be assembled to 487-fold sequence coverage. We found the *C. psittaci* genome being composed of a circular 1,171,660-bp chromosome containing a set of 967 predicted protein-coding genes and a 7,553-bp plasmid containing 8 protein-coding genes (Voigt *et al.* 2011).

As a first step towards examining the relative roles played by theses microbial genomes and the interaction between the pathogen and the host in causing differences in virulence or host preference, we compared the genome of the *Chlamydia psittaci* isolate with the fully sequenced genomes of several other chlamydial species, including *C. trachomatis*, *C. muridarum*, *C. pneumoniae*, *C. abortus*, *C. felis* and *C. caviae* (Voigt *et al.*, 2012).

This comparison revealed a high level of sequence conservation and synteny across taxa, with the major exception of the human pathogen *C. trachomatis*. Important differences mostly manifested in the *Chlamydiaceae*-specific polymorphic membrane protein (Pmp) family and in the highly variable chlamydial plasticity zone. Pmps are characterised by an unusually high level of mutational change within and between chlamydial species, and are present at hugely varying numbers across species (ranging from nine in *C. trachomatis* and *C. muridarum* to 21 in *C. psittaci*). This suggests fast evolutionary rates and high selective pressure potentially associated with adaptation to different hosts or immune responses. We identified a number of *C. psittaci*-specific polymorphic membrane proteins in the G family (Pmps cluster phylogenetically into 6 subfamilies, of which G is the most prolific) that may be related to differences in

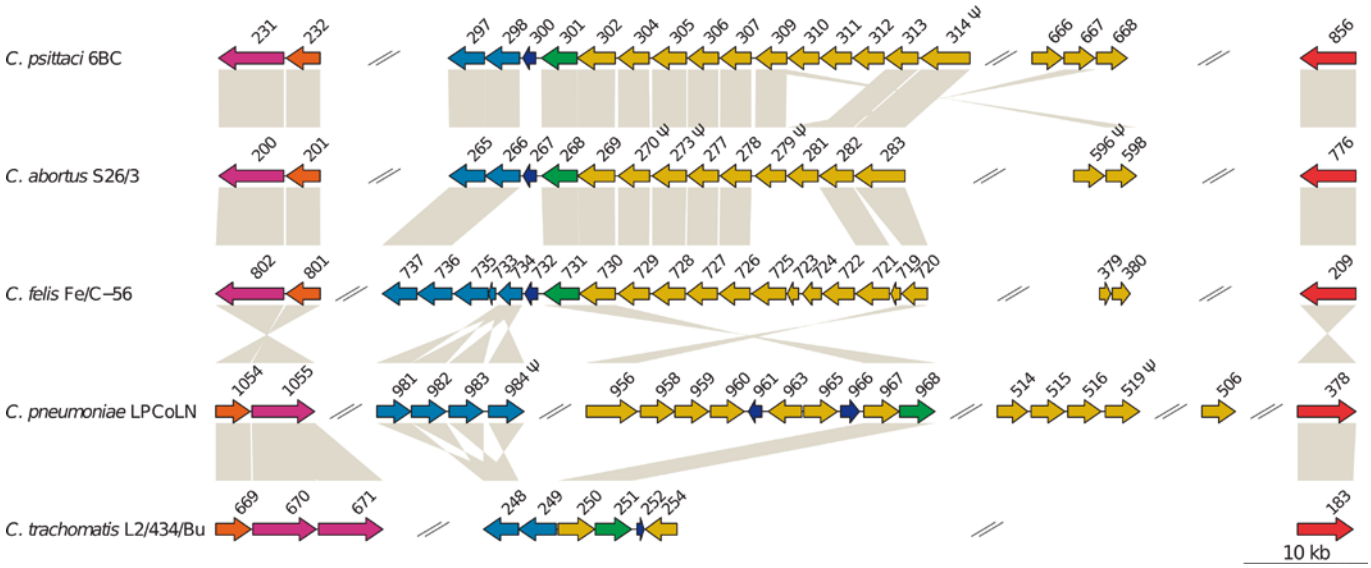

Figure 4
Organisation of the Pmp-family compared in *C. psittaci* 6BC, *C. abortus* S26/3, *C. felis* Fe/C-56, *C. pneumoniae* LPCoLN, and *C. trachomatis* L2/434/Bu. Arrows indicate the gene orientation. The colour code corresponds to the phylogenetic analysis of the Pmp-subfamilies: A (orange), B/C (purple), D (red), E/F (blue), G/I (yellow), and H (green). The open reading frame number is printed above the particular protein. Orthologous genes as inferred by phylogenetic analysis are connected by grey bars.

host-range and/or virulence as compared to closely related *Chlamydiaceae* (Figure 4).

To identify putative targets of adaptive evolution, we calculated non-synonymous to synonymous substitution rate ratios (d_N/d_s-ratios) for pairs of orthologous genes and predicted type III secreted effector proteins. The d_N/d_s-ratio is the basic measure of selective pressure acting on protein coding sequences across more distantly related taxa. Values below 1 suggest increasing evolutionary constraint. Not unexpectedly, most chlamydial genes were found to be subject to strong evolutionary constraints. As a notable exception, however, the comparison of *C. psittaci* with its closest relative *C. abortus*, showed markedly less overall level of genomic conservation than other comparisons within the same group of lineages and the highest overall variance in d_N/d_s-ratios. This comparison in fact yielded a number of candidate genes mostly of yet unknown function that are potentially under positive selection and might warrant further investigations.

To take these comparisons further and take advantage of population genetics methodologies, we recently added the genomes of four mammalian isolates of *C. psittaci* to the pool of chlamydial genomes (Schöfl *et al.*, 2011). This data-set is currently analysed for patterns of intraspecific variation and is hoped to help determining the genomic correlates of patterns of host adaptation and virulence within and among chlamydial species.

4 Analysis and visualisation of genome-wide DNA polymorphism

Group Leaders: Gerhard Schöfl, Hans Peter Saluz

An important limiting factor for the application of population genetics methods to com-

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plete genomes is the relative dearth of software tools that are up to that task. On the one hand software for estimating virtually every parameter or test ever conceived in population genetics abounds. On the other hand, these programs, however, were mostly designed in pre-genomics times for data sets composed either of a limited number of discrete genes or sets of specific genetic markers such as SNPs or AFLPs, and simply do not scale well to data sets of the size and complexity of multiple complete aligned genomes. Moreover few of these programs are capable to visualise results in an attractive fashion.

In the past years a number of population genetics statistics have been implemented in dedicated bioinformatics libraries such as BioPerl or BioPython. Their application, however, requires more than just basic programming experience by the user.
Motivated by our own needs for a tool to simultaneously visualise functional annotation information and the results of various test statistics as they change along a chromosome, we started to implement a suitable software package using the R environment for statistical computing and graphics.

R is an open source suite of software facilities for data manipulation, calculation and graphical display. It implements a huge number of statistical algorithms and, via its bioinformatics spin-off, the Bioconductor project, a number of data structures designed to facilitate manipulation of biological sequence information.

We have implemented methods for efficiently parsing a number of popular annotation data formats, such as GFF and GenBank files, and methods to adapt annotation information to multiple alignments of genomes. This allows to conduct global sliding-window-based analyses along chromosomes, to 'zoom' into specific regions or selectively extract regions with specific functional annotations of interest, and to visualise both the results of the analyses and the functional annotation at the analysed sites.

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

Group Leaders: Thomas Opfermann, Hans Peter Saluz

The aim of this project was to establish a molecular imaging technique combining positron emission tomography (PET) with computed tomography (CT) for investigating the pathogenicity of microbial infections and inflammations using established and new animal model systems. Because PET/CT is a non-invasive imaging method, stress on laboratory animals is heavily reduced (*refinement*). A *reduction* of animal experiments by using PET/CT is achieved because the same animal can be subjected to repeated measurements and can moreover act as its own control. Finally, by introducing the embryonated chicken egg as an alternative animal model, we aimed for the *replacement* of rodent experiments.

The project was a cooperation between the Department of Cell and Molecular Biology, the Institute of Molecular Pathogenesis (Friedrich Loeffler Institute, Jena), the Institute for Immunology (University Hospital, Jena), and the Department of Microbial Pathogenicity Mechanisms (Hans Knöll Institute, Jena). The following sub-projects were handled by the project partners:

1) Infection models for human pathogenic fungi (*Candida albicans* and *Aspergillus fumigatus*)
2) The embryonated chicken egg for the investigation of *Chlamydophila psittaci* infections
3) A mouse model for rheumatoid arthritis (Glucose-6-phosphate isomerase (G6PI) induced murine arthritis)

One of the main aims of our research was to establish the embryonated chicken egg as a model system for the investigation of infections caused by *C. albicans*, *A. fumigatus*, *and C. psittaci*. More specifically, our work involved developing anesthetic and injection

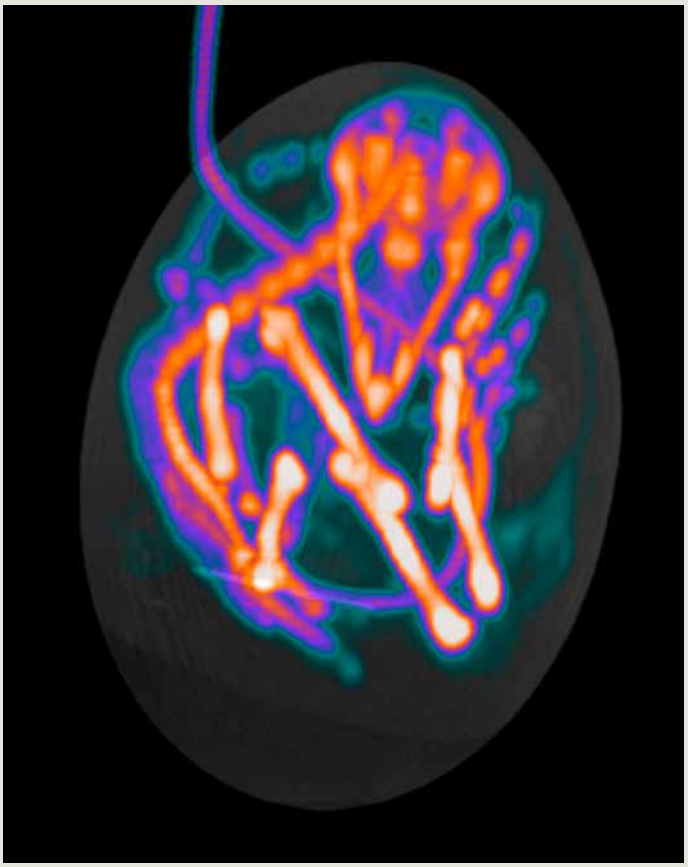

Figure 5
Imaging of the head and bones of embryonated chicken eggs by [^{18}F] Fluoride.

techniques. Furthermore, we developed and evaluated several radio tracers for the *in vivo* and *in vitro* detection of infections caused by the aforementioned pathogens. Finally, protocols for image data analysis were established and successfully applied.

A second focus of our work was the development and evaluation of different radio tracers for studying G6PI induced murine arthritis by PET/CT.

Techniques and technology

For the study of small animals and embryonated chicken eggs by PET/CT, optimising measurement conditions is an important point of consideration. First, long measurement times influence body temperature, which can potentially affect metabolic activity and thereby the experimental outcome. Furthermore, infected animals suffering from symptoms of systemic infection need special attention and careful handling. To account for these needs, the PET/CT scanner was equipped with a special measuring chamber (split top mouse chamber for Preclinical Imaging Systems, m2m imaging, USA). Additionally, we are developing a measurement chamber for optimising PET/CT measurements on embryonated chicken eggs.

For motion artifact–free PET and CT images, a method for anesthetising chicken embryos *in ovo* was developed (Heidrich *et al.*, 2011). Together with the refinement of intravascular tracer delivery, protocols for dynamic PET image analysis were developed. These techniques were used to study the *in ovo* bone metabolism of the developing chick embryo (Würbach *et al.*, 2012).

In vitro* test systems for *Aspergillus* and *Chlamydiae

For the investigation of tracer uptake of *A. fumigatus*, we adapted the LigandTracer (LT; Ridgeview Instruments AB, Sweden). The differences in tracer uptake of *Aspergillus*

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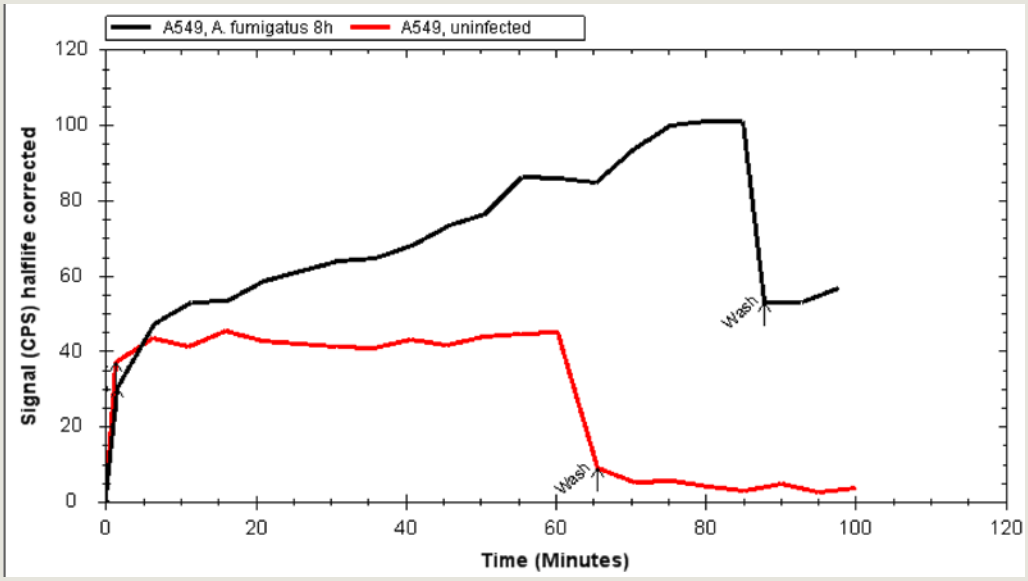

Figure 6
^{68}Ga-siderophore uptake studies on *Aspergillus* / cell line with the Ligand Tracer system

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strains (mutants) could be observed on the basis of the slope of the measurement curve as well as the absolute tracer uptakes.

Similarly, as in the experiments with human pathogenic fungi, we used the LT system for *Chlamydia* infected cells. Adherent cells were seeded on Petri dishes and infected with different multiplicity of infections (MOI). A glucose analog ([^{18}F]FDG) was added and by using the LT device, the specific real time uptake of the tracer could be observed. The slope of the resulting normalised uptake curves is dependent on the amount of infection.

Embryonated chicken eggs as a novel infection model for *Chlamydia psittaci*

After the introduction of this alternative *in vivo* model system, the application of [^{18}F]FDG and ^{89}Zr-labeled LPS antibodies for the detection of infection foci in embryonated chick eggs was investigated. Initial results revealed the suitability of these tracers for PET for *in vivo* imaging.

PET/CT in experimental arthritis

Murine models of rheumatoid arthritis (RA) may potentially reveal characteristics of human pathogenesis. In our studies, acute RA was induced in DBA/1 mice by immunisation with G6PI. Inflammation of joints was manifested by high accumulation of [^{18}F]FDG in joints. To extend the studies to immune cells, we labelled specific antibodies with the positron emitting ^{89}Zr. The tracer was injected into both mice with G6PI induced murine arthritis and control animals. Antibodies were cleared from the blood pool within 24 to 48 h. In control animals, the labelled antibodies accumulated in the spleen and lymph nodes (anti B220, anti CD4) or in the liver (anti G6PI and control/unspecific antibody). In animals with acute inflammation, increased uptake could be observed in the joints.

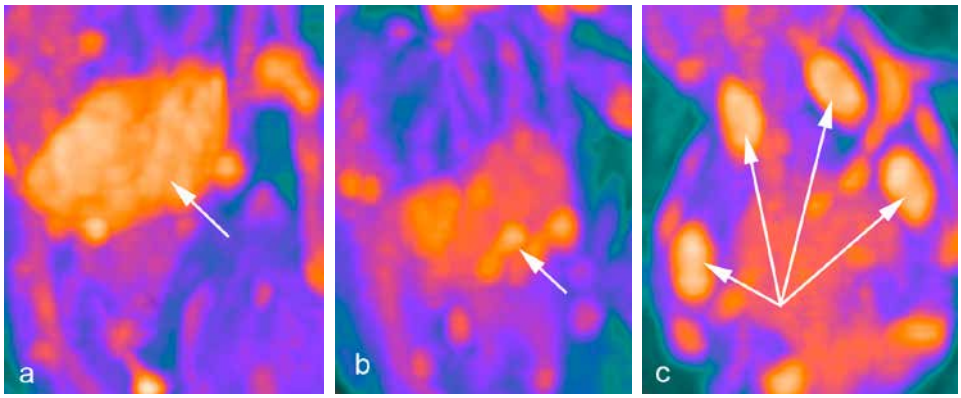

Figure 7
Uptake of [^{18}F]FDG in liver (A) and spleen (B) of infected eggs and joints (C) of the control.

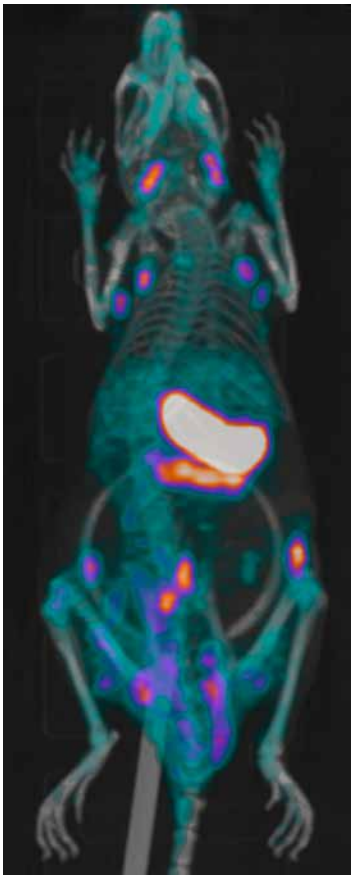

Figure 8
Accumulation of ^{89}Zr labeled anti-B220 antibodies in spleen and lymph nodes.

6 Functional Analysis of Cervimycin C resistance in *Bacillus subtilis*

Group Leader: Hans Krügel

To overcome increasing bacterial resistance to antibiotics, new compounds are being screened. One such compound, Cervimycin C (CmC), which belongs to a complex of compounds produced by *Streptomyces tendae*, consists of a tetracyclic polyketide substituted with chains of trideoxysugars and is only active against Gram-positive bacteria. To analyse the molecular response, we grew *Bacillus subtilis* in the presence of increasing CmC concentrations. Two independent CmC resistant clones of *B. subtilis* were identified, each carrying two mutations in the intergenic region upstream of the ABC transporter gene *bmrA*. Thus, the ABC-transporter confers antibiotic-resistance as a result of the cumulative effects of the two mutations in the promoter region. While such fast adaptation to antibiotic stress limits their usefulness as new drugs, many antibiotics may still become important tools for elucidating the biology of microorganisms. Currently, another mutation conferring resistance by a different mode is being investigated.

Small non-coding RNAs (sRNAs) regulate gene expression in all three kingdoms of life. So far, relatively little is known about sRNAs from Gram-positive bacteria. SR1 is a regulatory sRNA from the *B. subtilis* chromosome that, through base-pairing, inhibits translation initiation of *ahrC* mRNA, which encodes a transcriptional activator of the arginine catabolic operons. Here we present a novel target of SR1, the glycolytic *gapA* operon. Both microarray and Northern blot analyses show that the amount of *gapA* operon mRNA is significantly higher in the presence of SR1 when cells were grown in complex medium until stationary phase. Translational *lacZ* fusions and toeprinting analyses demon-

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strate that SR1 does not promote translation of *gapA* mRNA. In contrast, the half-life of *gapA* operon mRNA is strongly reduced in the *sr1* knockout strain. SR1 does not act as a base-pairing sRNA on *gapA* operon mRNA. Instead, we demonstrate that the 39- amino acid peptide encoded by SR1, SR1P, is responsible for the effect of SR1 on the *gapA* operon. We show that SR1P binds GapA, thereby stabilizing the *gapA* operon mRNA by a hitherto unknown mechanism. SR1 is the first dual-function sRNA found in *B. subtilis*.

7 Antibiotic resistome in the food chain

Group Leader: Hans Krügel

The increasing use of antibiotics in farm animals leads to the spread of antibiotic-resistant microorganisms. Therefore, we analysed the distribution of antibiotic resistance genes in microorganisms at various points in the food chain. We especially focused on the presence of mobile genetic elements, which allow the transfer of pathogens from commensals to humans. Moreover, members of genera that are pathogenic to humans were investigated (e.g., *Salmonella*, *Klebsiella*, *Pseudomonas*, *Staphylococcus*, *Acinetobacter*, *Escherichia*). In raw food (e.g., meat), more bacteria were found than in cooked food. Isolates resistant to multiple antibiotics (e.g., penicillin, sulfonamide and tetracycline) were widespread. The most frequently occurring genes were *tetA*, *tetB*, *aadA1*, *aph1*, *bla-TEM*, *bla-ampC*, *sul1* und *sul2*. Integrons were often detectable and contained *dhfrA1*, *sat2* und *aadA1*. A more detailed analysis of one plasmidome was initiated.

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

Group leader: Hans Peter Saluz
(in cooperation with (GMBU) Jena)

As part of a comprehensive multicolour genotyping microarray research programme, we have developed in cooperation with Gesellschaft zur Förderung von Medizin-, Bio- und Umwelttechnologien e.V. (GMBU), Jena, a two-laser large field hyperspectral multicolour microarray scanning system. 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.

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PET-CT – PET/CT zur Darstellung von Infektion und Entzündung: Reduction, Refinement und Replacement von Tierversuchen
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PET-CT II – *In-vivo*-Quantifizierung von Inflammation und Destruktion in murinen Arthritismodellen mittels PET/CT als Beitrag zur Reduktion der Zahl der in Forschung und Entwicklung verwendeten Versuchstiere
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Research Group Applied Systems Biology

Research Group Applied Systems Biology

The Research Group Applied Systems Biology (ASB) was newly established at the Hans Knöll Institute (HKI) in 2011. The focus of this group is on the mathematical modelling and computer simulation of dynamical, functional and morphological aspects of infection processes. The theoretical studies are performed in collaborations with experimentalists at the HKI and beyond.

Today, a vast amount of image data is generated by microscopy experiments and the general experience that *"a picture is worth a thousand words"* also holds in the field of Systems Biology. Image data represent a firm data basis that contains essential information on spatio-temporal aspects of infection processes. The connecting link in joint studies of experiment and theory is referred to as *Image-based Systems Biology* and involves three main aspects: (i) automated analysis of image sequences for high-content screening of large data sets, (ii) quantification of biological processes involving mathematical analyses of the evaluated image data, and (iii) integration of spatio-temporal information into models that can be simulated on the computer to generate testable predictions and hypotheses.

Image-based Systems Biology is realised in the Research Group ASB with regard to all three aforementioned aspects. Fully automated analysis of image data is performed by Franziska Mech (Ph.D. student of the research group ASB) who is developing

INTRODUCTION | EINLEITUNG

Head:
Prof. Dr. Marc Thilo Figge

Die Forschungsgruppe Angewandte Systembiologie (ASB) wurde im Jahr 2011 am Hans-Knöll-Institut (HKI) etabliert. Der Fokus dieser Gruppe ist die mathematische Modellierung und Computersimulation dynamischer, funktionaler und morphologischer Aspekte von Infektionsprozessen. Diese theoretischen Studien werden in Zusammenarbeit mit Experimentatoren am HKI und darüber hinaus durchgeführt.

Eine umfassende Menge an Bilddaten wird heutzutage in Mikroskopie-Experimenten gewonnen und die allgemeine Erfahrung, dass „ein Bild mehr als tausend Worte sagt", gilt auch im Bereich der Systembiologie. Bilddaten stellen eine solide Datenbasis dar, welche wichtige Informationen zu raum-zeitlichen Aspekten von Infektionsprozessen enthält. Die *Bildbasierte Systembiologie* stellt ein Bindeglied in kombinierten Studien von Experiment und Theorie dar und umfasst die folgenden drei Hauptaspekte: (i) automatisierte Analyse von Bildsequenzen für die Hochdurchsatz-Untersuchung großer Datensätze, (ii) Quantifizierung biologischer Prozesse mit Hilfe mathematischer Analysen der ausgewerteten Bilddaten und (iii) Integration der raum-zeitlichen Information in Modelle, die auf dem Computer simuliert werden, um überprüfbare Voraussagen und Hypothesen zu generieren.

In der Forschungsgruppe ASB wird die *Bildbasierte Systembiologie* hinsichtlich aller drei genannten Aspekte realisiert. Die vollautomatische Analyse von Bilddaten wird von Franziska Mech (Doktorandin der Forschungsgruppe ASB) realisiert, wobei Regelsätze erstellt und in Algorithmen übersetzt

software based on rule-sets of image analysis within the framework of the commercial software *Definiens Developer XD®*. In joint experimental and theoretical studies, this approach was successfully applied in collaboration with the Department of Molecular and Applied Microbiology (Prof. A. Brakhage) regarding the phagocytosis of *Aspergillus fumigatus* by macrophages and is currently applied to quantify the dynamics of morphological changes during the invasion of epithelial cells by *Candida albicans* in collaboration with the Department of Microbial Pathogenicity Mechanisms (Prof. B. Hube). Moreover, going beyond the application of commercial software, Dr. Carl Svensson (PostDoc of the research group ASB) is developing unsupervised machine learning methods for the automated analysis of image sequences. This approach is developed in collaboration with the research group Computational Neuroscience and Machine Learning of Dr. J. Lücke from the Frankfurt Institute for Advanced Studies (FIAS) at the Goethe University Frankfurt. In the future, automated image analysis will become independent of using commercial software by developing state-of-the-art methods from the field of unsupervised machine learning.

The second aspect of *Image-based Systems Biology* is covered by the work of Zeinab Mokhtari (Ph.D. student of the research group ASB) who applies mathematical concepts to derive characteristic quantities from the raw data resulting from the image

werden, um mittels der kommerziellen Software *Definiens Developer XD®* auf die Bilddaten angewandt zu werden. In kombinierten Studien von Experiment und Theorie wurde dieser Ansatz erfolgreich realisiert, beispielsweise in Zusammenarbeit mit der Abteilung Molekulare und Angewandte Mikrobiologie (Prof. A. Brakhage) für die Untersuchung der Phagozytose von *Aspergillus fumigatus* durch Makrophagen. In Zusammenarbeit mit der Abteilung Mikrobielle Pathogenitätsmechanismen (Prof. B. Hube) wird auf diese Weise die Dynamik morphologischer Änderungen von *Candida albicans* während der Invasion von Epithelzellen quantifiziert. Über die Anwendung kommerzieller Software hinausgehend entwickelt Dr. Carl Svensson (PostDoc der Forschungsgruppe ASB) Methoden des unüberwachten maschinellen Lernens zur automatisierten Analyse von Bildsequenzen. Dieser Ansatz wird in Zusammenarbeit mit der Forschungsgruppe Rechnergestützte Neurowissenschaften und Maschinelles Lernen von Dr. J. Lücke am Frankfurt Institute for Advanced Studies (FIAS) an der Goethe Universität Frankfurt verfolgt. In Zukunft soll die automatisierte Bilddatenanalyse durch die Entwicklung moderner Methoden des unüberwachten maschinellen Lernens unabhängig von der Anwendung kommerzieller Software werden.

Der zweite Aspekt der *Bildbasierten Systembiologie* wird von Zeinab Mokhtari (Doktorandin der Forschungsgruppe ASB) bearbeitet, wobei mathematische Methoden zur Anwendung kommen, um charakteristische Kenngrößen aus den Rohdaten der Bilddatenanalyse zu ermitteln. So erlauben es beispielsweise Multiphotonenmikrosko-

analysis. For example, multi-photon microscopy experiments allow for recording the dynamics of biological processes, e.g. by tracking of single cells *in vivo*. The computation of quantities, such as the motility coefficient, is obtained as the result of averaging over cellular ensembles by which the absolute positions of cells in the biological sample are neglected. As it turns out that cells may change their migration behavior depending on their absolute position in the biological sample, it is important to also quantify cellular dynamics at the single-cell level. Developing mathematical methods that satisfy these constraints is performed in the research group ASB. *In vivo* cell track data are generated by multi-photon microscopy experiments and are made available by Dr. J. Stein of the research group Lymphocyte Trafficking and Activation at the Theodor Kocher Institute in Bern, Switzerland, and by Prof. Dr. A. Hauser of the research group Immunodynamics at the German Rheumatism Research Centre Berlin.

Finally, the third aspect of *Image-based Systems Biology* concerns the integration of the quantified spatio-temporal information into models that can be simulated on the computer to generate testable predictions and hypotheses. The adequate method to achieve this goal involves the application of the agent-based modelling approach, which is realised by Johannes Pollmächer (Ph.D. student of the research group ASB and JSMC fellow) and Teresa Lehnert (Ph.D. student of

pie-Experimente die Dynamik biologischer Prozesse einzufangen, indem die Migration einzelner Zellen *in vivo* verfolgt wird. Die Berechnung von charakteristischen Kenngrößen, wie beispielsweise dem Motilitätskoeffizienten, ist das Resultat einer Mittelung über die Gesamtheit aller Zellen unter Vernachlässigung ihrer absoluten Position im biologischen System. Da Zellen ihr Migrationsverhalten jedoch abhängig von ihrer absoluten Position im biologischen System verändern können, ist es entscheidend, die zelluläre Dynamik auch auf der Ebene einzelner Zellen zu untersuchen. Die Entwicklung entsprechender Methoden wird in der Forschungsgruppe ASB betrieben. *In vivo* Zelltrack-Daten werden mittels der Multiphoton Mikroskopie von Dr. J. Stein der Forschungsgruppe Migration und Aktivierung von Lymphozyten am Theodor Kocher Institut in Bern, Schweiz, sowie von Prof. Dr. A. Hauser der Forschungsgruppe Immundynamik des Deutschen Rheuma-Forschungszentrums Berlin gewonnen und zur Verfügung gestellt.

Der dritte Aspekt der *Bildbasierten Systembiologie* betrifft die Integration der quantitativen raum-zeitlichen Information in Modelle, die auf dem Computer simuliert werden und überprüfbare Voraussagen und Hypothesen generieren können. Um diesem Anspruch gerecht zu werden, erweist sich der Agentenbasierte Modellierungsansatz als die angemessene Methode und wird von Johannes Pollmächer (Doktorand der Forschungsgruppe ASB) und Teresa Lehnert (Doktorandin der Forschungsgruppe ASB) angewandt. Der Agentenbasierte Modellierungsansatz betrachtet Zellen als individuelle und dis-

the research group ASB). In the agent-based approach, cells are treated as individual and discrete objects that are migrating and interacting in space and time. Using the data basis that is obtained as a result of image analyses and subsequent mathematical evaluations, the stochastic dynamics of the biological system can be mimicked at realistic levels. Unknown parameters, e.g. regarding the interaction between cells, can be adjusted, e.g. by comparing differences in the behavior between wild type and mutants, in order to generate predictions that can be further tested in experiment. Currently, we are at the stage of implementing agent-based models for large-scale multi-cellular systems of infection processes as well as molecular transmembrane signalling during the interaction of single cells. Setting up a generic framework at both these levels will allow performing comprehensive computer simulations for different experimental systems in the future. This will enable us to investigate aspects with regard to dynamics, function and morphology of host-pathogen interactions by *Image-based Systems Biology*.

krete Objekte, welche sich in Raum und Zeit bewegen und miteinander interagieren können. Aufbauend auf der Datenbasis, welche durch die Bilddatenanalyse und die anschließende mathematische Evaluierung gegeben wird, kann die stochastische Dynamik des biologischen Systems in realistischer Weise nachgestellt werden. Unbekannte Parameter – z. B. im Blick auf die Wechselwirkung zwischen Zellen – können angepasst werden – z.B. durch den Vergleich von Unterschieden im Verhalten zwischen dem Wildtyp und Mutanten – mit dem Ziel, Voraussagen zu generieren, die in weiteren Experimenten getestet werden können. Die Implementierung Agentenbasierter Modelle für die Simulation von Zellsystemen muss für große Anzahlen unterschiedlicher Zellen und für die Simulation von Signalübertragungen zwischen einzelnen Zellen unter Berücksichtigung der großen Zahl an interagierenden Oberflächenmolekülen ausgelegt werden. Der Aufbau von generischen Umgebungen auf diesen beiden Ebenen wird es in Zukunft möglich machen, umfassende Computersimulationen verschiedener experimenteller Systeme durchzuführen. Diese können dann dafür genutzt werden, Phänomene bezüglich dynamischer, funktionaler und morphologischer Aspekte der Wirt-Pathogen-Interaktion mit Hilfe der *Bildbasierten Systembiologie* zu untersuchen.

1 Automated image analysis of host-pathogen interactions

Group Leaders: Franziska Mech, Carl Svensson, Marc Thilo Figge

Interaction between phagocytes and *Aspergillus fumigatus*

Sophisticated microscopy techniques combined with automated image analysis are a powerful tool in biology. We employed this procedure to study infection processes with the fungus *Aspergillus fumigatus*. *A. fumigatus* produces conidia during asexual reproduction as the common reproductive form. Every day healthy humans inhale hundreds of conidia without getting infected. By contrast, in immunocompromised patients *A. fumigatus* can cause invasive pulmonary aspergillosis (IPA) that results in mortality rates of about 30–95 %. Therefore, fungal infections and their impact on the human immune system are a major issue in current research and the comparative analysis of various mutants is of great importance to get deeper insight into the pathogenicity mechanisms of this fungus. Since the proper recognition, adherence and ingestion of inhaled conidia by phagocytes represent critical steps in the infection process of *A. fumigatus*, the determination of phagocytosis ratios provides crucial insights into infection mechanisms.

In the present phagocytosis assays, cells were differentially stained with fluorescent dyes and visualised with a confocal laser scanning microscope, leading to the discrimination between macrophages, internalised conidia and non-internalised conidia. We performed an automated image analysis which is a challenging task for the following reasons: (i) conidia and macrophages are often clustered and attached to each other, (ii) both cell types show variations in their internal and in-between intensities, and (iii) the background is variable. The automated image analysis was realised by applying the commercial software *Definiens Developer XD*®, which requires writing of an algorithm in a meta-language that is based on a ruleset. The developed ruleset allows for the segmentation and classification of the regions of interest (ROI), being the image objects composed of macrophages and conidia. It turned out that only a few input-parameters in terms of threshold values are required, i.e. for the typical object areas, for their roundness and for their intensity. The method is able to identify conidia properly, even if they are clustered or have an asymmetrical shape. A rigorous validation of the segmentation and classification results showed that the ruleset was able to clearly differentiate between macrophages and the different types of conidia. Therefore, we demonstrated that our method yields correct image analysis compared with a manual procedure accompanied by a drastically reduced expenditure of time.

The statistical analysis of the phagocytosis ratio confirmed the results of previous experiments showing a significantly increased phagocytosis of *pksP* mutant conidia in contrast to the wild type. This is due to an increased amount of β1-3glucan displayed on the surface of resting *pksP* mutant conidia by loss of a functional melanin layer, thereby enhancing phagocytosis mediated by the C-type lectin-like surface receptor dectin-1. Furthermore, our analysis revealed that the *pksP* mutant conidia besides an altered uptake ratio by macrophages also show increased conidial aggregation behavior compared with the wild type. During germination, conidia undergo a drastic remodelling of the conidial cell wall. This includes loss of the outer rodlet/melanin cell wall layer, leading to the exposure of the inner cell wall layer. Since resting *pksP* mutant conidia display an increased amount of β1-3glucan exposed on the conidial surface, it is reasonable to assume that the accessi-

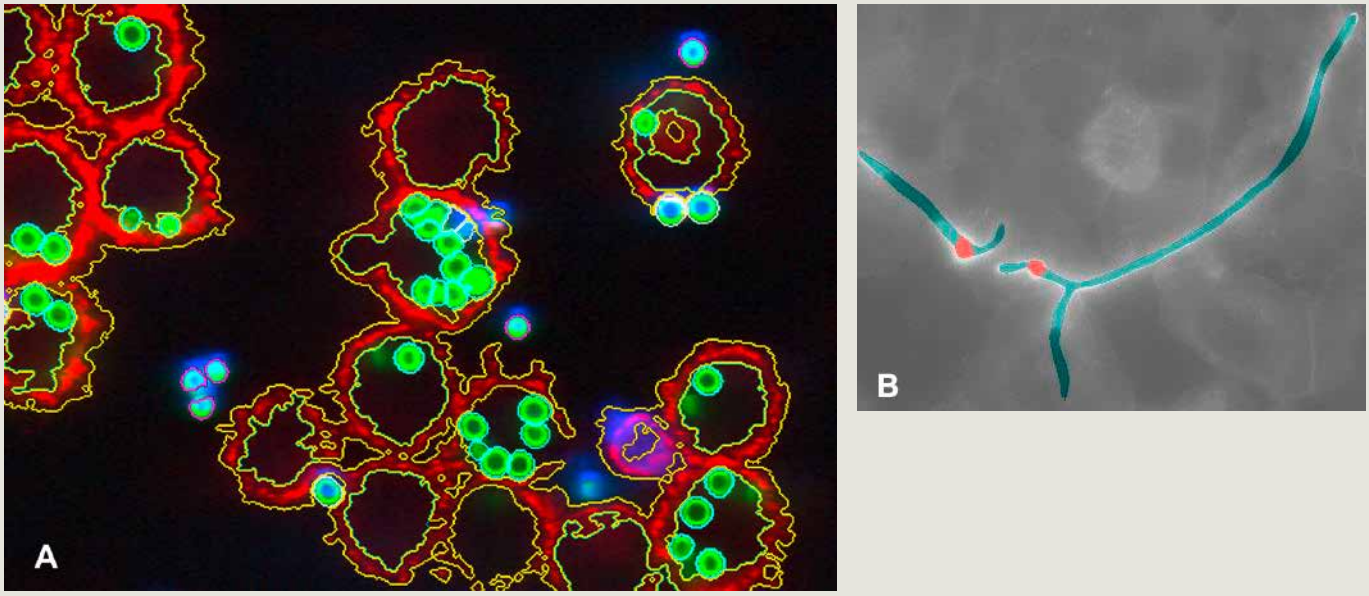

Figure 1
(A) Segmentation of macrophages (red) and *Aspergillus fumigatus* conidia that were phagocytozed (green) or not detected (blue) by automated image analysis. **(B)** Segmentation of *Candida albicans* invasion with hyphae germinating from yeast cells (red) being either outside (light green) or inside (dark green) epithelial cells (grey) by automated image analysis.

bility of ß1-3glucan on resting *pksP* mutant conidia is also higher than on wild-type conidia. Thus, *pksP* mutant conidia do not only have an increased phagocytosis ratio as compared to wild-type conidia but also show significantly increased aggregation behavior. Previous experimental setups did not allow for studying these morphological aspects in detail, since they can only be obtained as the result of analyzing the behavior of individual cells in high-throughput microscopy measurements.

The developed ruleset for processing microscopic raw data is easy to implement and allows for the fully automated and context-based analysis of image data. The image analysis is suitable to obtain reproducible results from spatially resolved biological data and to replace tedious and error-prone manual work. High precision rates and classification scores show that this method has the potential to be used in high-throughput microscopy measurements and may not only be applied to other strains of *A. fumigatus* but as well to other fungal species. Since conidia, or any small round cells, represent the infectious agents of many diseases, the understanding of the recognition and phagocytosis process can contribute to a better understanding of the virulence process of other relevant pathogens like *Candida spp.*, *Cryptococcus neoformans* or *Histoplasma capsulatum*. Therefore, the developed ruleset will contribute to improve the quantity and quality of phagocytosis analyses in general. The fast analysis and statistical evaluation of features from large amounts of data are also required in the context of developing spatio-temporal *in silico* models of the host-pathogen interaction.

Invasion of epithelial cells by *Candida albicans*

Under normal circumstances, *Candida albicans* is a harmless commensal of the gastrointestinal and genitourinary tract. However,

this fungus can turn into a dangerous pathogen causing severe infections, especially in immunocompromised patients, patients suffering from human immunodeficiency virus (HIV), intensive care patients that undergo cancer chemotherapy or immunosuppressive therapy after organ or bone marrow transplantation. In order to invade host-cells and to gain access to the bloodstream, *C. albicans* produces filamentous hyphae for the invasion of epithelial barriers. The progression of hyphae formation is associated with distinct stages of infection starting with the adhesion of *C. albicans* to host cells, subsequent invasion of epithelial cells by hyphae of the fungus and, finally, damaging of the tissue.
In this project we started to investigate the kinetics of morphological changes of *C. albicans* during the first six hours after infection of epithelial cells. We obtained image data from fluorescence microscopy experiments at one-hour intervals that monitor yeast cells of *C. albicans* on epithelial cells starting to form hyphae and invading the host cells. Initially, all yeast cells were fluorescently labeled and re-stained with a different colour before taking the microscopy images. In this way, it is possible to distinguish those parts of a hypha that are inside epithelial cells from parts that are outside or lying in between epithelial cells.

We have started to apply image analysis by identifying the ruleset for an algorithm to be run by the software *Definiens Developer XD*® that will allow quantifying the morphological kinetics of *C. albicans* during the infection of epithelial cells in a fully-automatised fashion. At each time point, quantities of interest include the hypha length, the number of invasions of epithelial cells, the number of secondary hyphae formation of a yeast cell, the occurrence of branches along hyphae etc. The collection of these characteristic quantities will contribute to our understanding of the time-evolution of morphological changes during infection processes.

2 Mathematical analysis of single cell dynamics from image data

Group Leaders: Zeinab Mokhtari, Marc Thilo Figge

Today, the state-of-the-art technique for cell tracking in living systems is multiphoton microscopy, where the position of single cells is recorded in time yielding a four-dimensional data set for each monitored cell. The data contain essential information on the migration and interaction of cells in biological systems that may depend on their absolute position in space. Characteristic quantities that are typically calculated include speed and turning angle distributions as well as displacement curves that allow distinguishing directed cellular migration from random walk behavior. However, the calculation of these quantities involves averaging over the cellular ensemble by which the information on the cellular behavior as a function of its absolute position in the biological sample gets lost. Therefore, restriction of the analysis to average quantities does not do justice to the detailed measurements as performed by multiphoton microscopy.

We addressed this issue by mathematical tools that enable us to analyze properties of single cells as a function of their absolute position in space. Thus, retaining the full four-dimensional information as obtained from multiphoton microscopy for single cells, we showed that the time-dependence of a cell track in three-dimensional space could be translated into a set of three simple curves as a function of time. This was achieved by representing cell tracks within matrix formalism, where the transition matrix between subsequent time points was proven to be composed of a scalar operation and a rotation operation. After translating the cellular track into the corresponding product of transition matrices, we found the displacement vector of the cell to be encoded in the three eigenvalues of the resulting matrix. Most importantly, the migration behavior of single cells can be interpreted by inspecting

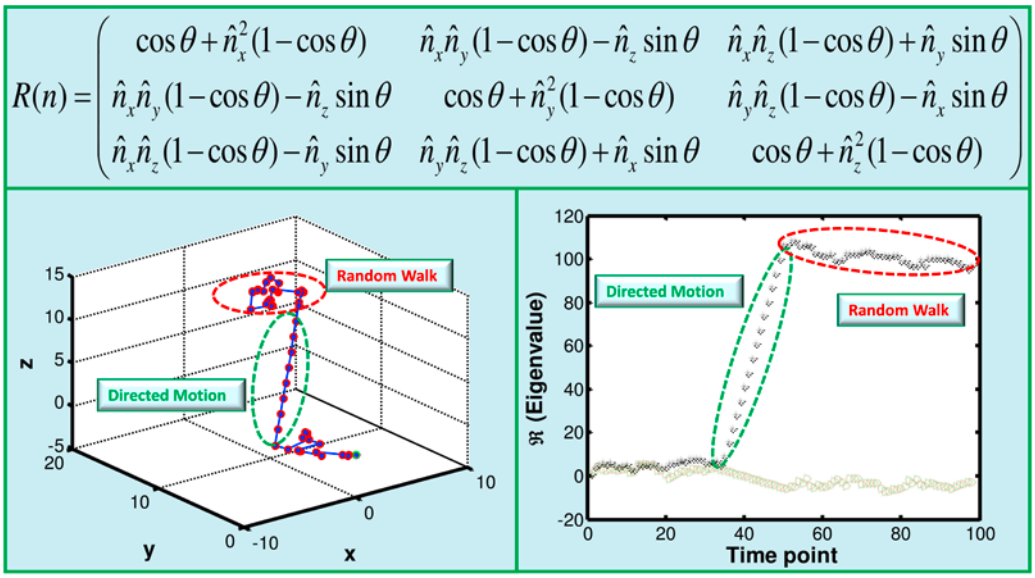

Figure 2
The time-evolution of the cellular path in three spatial dimensions is translated into the eigenvalues of the transition matrix containing the same information that allows distinguishing directed motion from a random walk of the cell.

the evolution of eigenvalues as a function of time in a rather simple manner. To fully exploit this approach we are currently developing algorithms for the classification of the dynamically changing cellular migration behavior in biological systems.

The importance of single cell track analysis was previously pointed out by us with regard to B lymphocyte migration in germinal centers. Cell tracks were obtained from multiphoton microscopy experiments and the calculation of their displacement curve as a function of the square-root of time suggested that B lymphocytes perform random walk migration in germinal centers. It could be shown, however, that this conclusion cannot be reconciled with the morphology of germinal centers. If B lymphocytes were to perform random walk migration throughout the germinal center, then the experimentally observed separation of germinal centers into a distinct dark and light zone would not be present. This inconsistency could be resolved by the prediction that B lymphocytes are predominantly performing random walk migration within the dark and light zone, however, at the interface of the two zones their migration behavior becomes altered due to transient chemotaxis. All experimental data could be reconciled by suggesting that the migration behavior of B lymphocytes depends on the absolute position in germinal centers. This study indicated that the transient migration behavior of single cells can get lost by computing characteristic quantities from averages over the cellular ensemble in which the majority of cells show a different migration behavior. Recently, we performed single cell analyses for B lymphocytes that are homing to follicles as observed by multiphoton microscopy in murine lymph nodes. In this case it was expected to find that B lymphocytes are directed by chemotactic signals towards the follicles. However, as we could show by a single cell analysis

of these cell tracks, rather than undergoing chemotaxis, B lymphocytes undergo chemokinesis by increasing their speed.

Taken together this project serves the general need to provide mathematical analyses of single cell dynamics that include all details of information as acquired in multiphoton microscopy experiments.

3 Agent-based modelling and computer simulations of infection processes

Group Leaders: Johannes Pollmächer, Teresa Lehnert, Marc ThiloFigge

We started to set up a computational framework that is widely applicable to model infection processes at different levels of complexity. This involves a separation of scales for which experimental data are becoming available and can be integrated into the models to simulate infection processes. In the first setup we focus on systems that contain a large number of migrating and interacting cells, whereas in the second setup we consider transmembrane signalling at the level of cellular surface molecules in the cellular interaction synapse. The two setups have in common that they are dealing with biological systems containing a large number of constituents, i.e. either the number of cells or the number of cellular surface molecules range up to tens of thousands. This requires applying sophisticated algorithms in order to keep simulations computationally feasible and is realised within the agent-based modelling approach.

Agent-based models treat cells and molecules as what they really are – discrete and individual objects in space and time. Each cell or molecule in the model is treated as an individual agent with a defined position in space and time. These agents are characterised by specific rules that are implemented in the model and reflect the properties of their real biological counterparts. For example, the speed of immune cells or their rate of phagocytosis as determined from the image analysis of time-lapse microscopy experiments are incorporated into the model by rules such that agents are adjusted to reproduce the same dynamic behavior in the simulations. To make the simulations as realistic as possible, we have chosen to implement the agent-based model in continuous space, i.e. agents can take on any position in space instead of being restricted in their dynamics by an artificial spatial grid.

The combination of a continuous space representation and a large number of interacting agents poses a critical situation with regard to computational feasibility. In contrast to grid models, where the neighborhood of agents is uniquely defined by the surrounding grid points such that the computing time for interacting agents scales linear with their number, in the continuous space representation the computing time increases with the square of the agent number. This is a consequence of the fact that per time step and for each agent we first have to identify its neighboring agents from all the other agents in order to identify possible interaction partners. To decrease the computing time in the continuous space representation we implemented a neighbor-list method. We proved that the performance of our algorithm with continuous space representation was greatly increased yielding computing times that are nearly back to the linear dependence on the agent number. Our neighbor-list method yields a speed up of computing time at the cost of computer memory, however, in contrast to the computing time the computer memory does not impose limitation for today's workstations.

In brief, the neighbor-list method can be envisaged as an artificial grid that is implemented in addition to the continuous space in which agents are migrating and interacting. Each grid point of the artificial grid owns a bookkeeping list that is associated with a particular region in continuous space and contains all agents in that region by

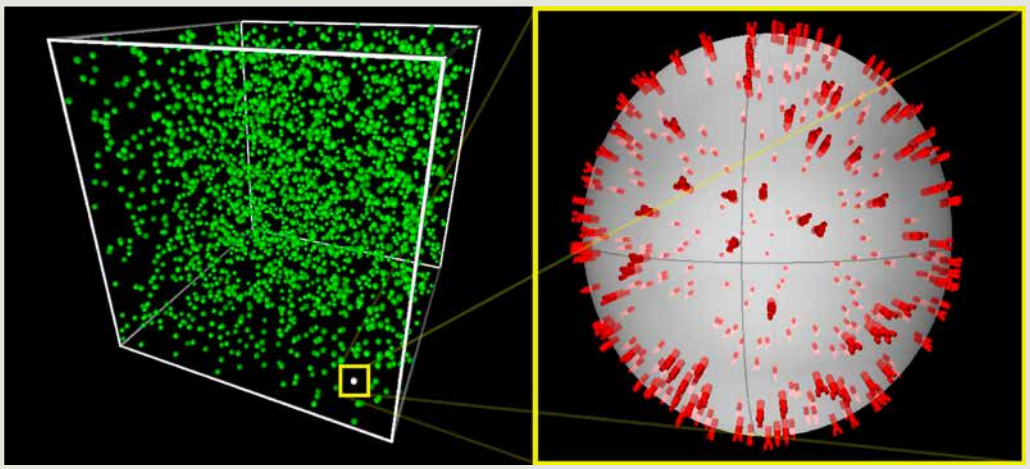

Figure 3
Agent-based modelling of infection by human-pathogenic fungi both at the level of systems containing a large number of migrating and interacting cells as well as at the level of cellular surface molecules in the interaction synapse of single cells.

their identification number. We found that the optimal region of each grid point is determined by the largest agent diameter, in which case any given agent can only be in contact with all agents that are associated with neighboring grid points of the one associated with the agent under consideration. It should be noted that this method is very effective because in biological systems interactions between agents are typically short-ranged since they depend on physical contact between the interaction partners.

Next, we investigated the additional gain in reducing the computing time by a parallelization of the algorithm. As the computing time was already linear in the agent number, parallelization of the algorithm could only reduce the average computing time that is spent per agent in each time step. Parallelization of the algorithm could be realised in a way that no direct communication between processors acting in parallel does exist. However, since system borders have to be treated with care and have to obey the boundary conditions, we found that the distribution of the workload between processors is a critical issue and has to be adjusted in order to reduce the waiting times between the processors that refer to the boundary and the bulk. Thus, the number of processors and the distribution of the workload required careful tailoring to the system under consideration.

Another important issue of the computer simulations is related to data presentation. We store system configurations at each time step and visualise the data, e.g. by performing snapshots and movies of the simulations. Furthermore, on the basis of the stored system configurations, we analysed the data by applying mathematical concepts that were developed in other projects. Thus, so far, we have implemented an agent-based model that was rigorously tested by performing numerous computer simulations and that will be

applied to model infection processes of human-pathogenic fungi both at the level of systems containing a large number of migrating and interacting cells as well as at the level of surface molecules in the interaction synapse of single cells.

4 Modelling the dynamics of interaction networks for immune responses

Group Leaders: Teresa Lehnert, Marc Thilo Figge

A computational framework was developed that allows modelling the dynamics of large-scale interaction networks by a set of coupled differential equations. This approach is suitable to describe the kinetics of populations, e.g. the concentrations of immune cells during an immune response, and applies to situations where the experimental distribution of the interacting constituents is spatially homogenous. Thus, based on the law of mass action, differential equations describing the time-evolution of the populations are obtained from the corresponding chemical reaction equations.

We implemented an algorithm that performs the time-integration of large-scale sets of coupled differential equations by the fourth-order accurate Runge-Kutta scheme. In order to carry out the time-integration, quantitative values for the reaction rates have to be provided. However, these values are often unknown *a priori* and are to be inferred from the solution of the dynamic interaction network by comparison with experiment. To achieve this goal we apply the following strategy: Starting from an initial set of reaction rates, which may be chosen at random within reasonable physiological limits, we compute the system's time-evolution and compare the result with the experimentally measured population kinetics. This is done by calculating deviations between computed and measured data. The resulting error function is minimised by randomly adapting reaction rates and by applying least error-square techniques to decide about accepting the new set of reaction rates.

The minimization of the error function in the high-dimensional parameter space is performed by the method of simulated annealing. In practice, the new set of parameters will be always accepted if it gives rise to an improved fit of the population kinetics to the experimental data. However, since this may yield a solution that is trapped in a local minimum of the parameter space, we compute a probability for accepting a new set of parameters even if the error function is not minimised with regard to the previous set of parameters. This ensures that the system can deviate from local minima in the parameter space and ultimately find the optimal set of parameters. The acceptance probability depends exponentially on the corresponding error-square such that larger deviations from a minimum are more unlikely to occur. This so-called Metropolis Monte Carlo approach results in the equilibration of the system after a sufficiently large number of trials for which the set of parameters eventually minimises the error function at the global minimum.

We started to test this approach by modelling the dynamic immune response to *Candida albicans* in human full-blood samples as measured by fluorescence activated cell sorting (FACS) analysis during the first four hours of infection. The interaction network was established for the measured population kinetics of granulocytes and monocytes during phagocytosis of *C. albicans* as well as for the population kinetics of yeast cells surviving phagocytosis. The differential equations account for the uptake of yeast cells by immune cells and for their subsequent killing with separate reaction rates. Furthermore, we take explicitly into account that immune cells which phagocytozed yeast cells for the first time subsequently respond with higher uptake and killing rates. In the interaction network, granulocytes and monocytes are treated in a *qualitatively* symmetric manner,

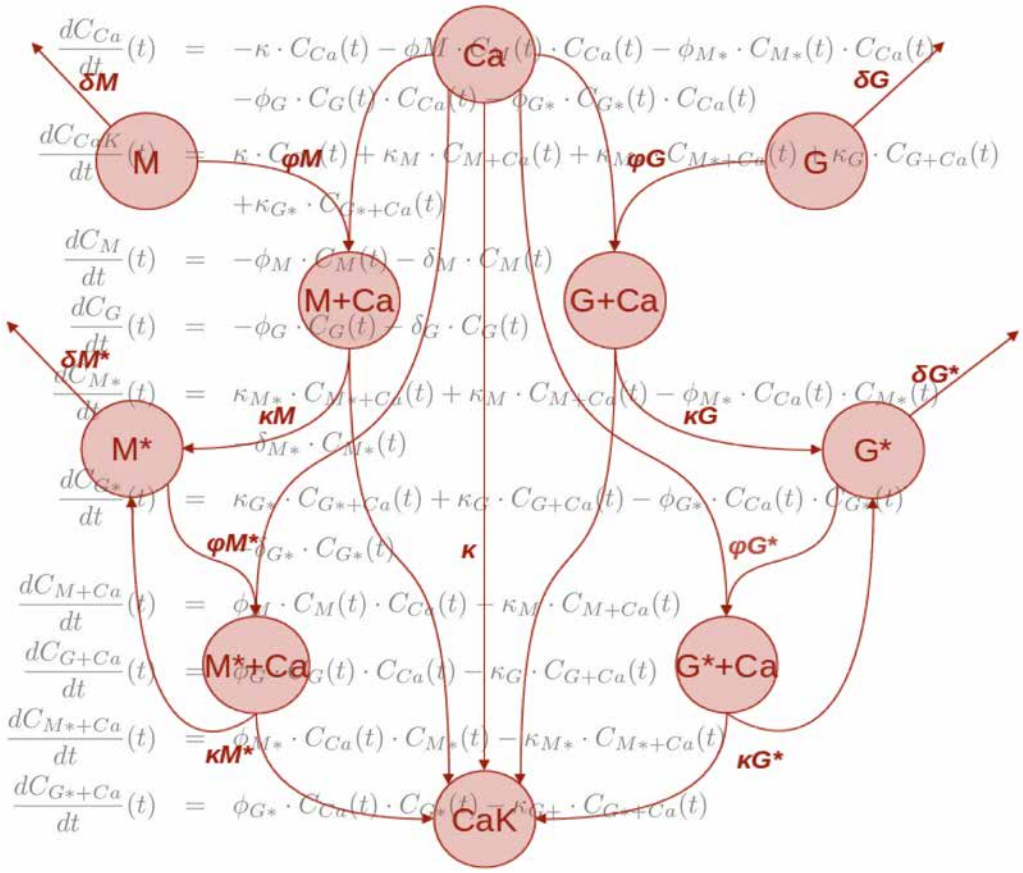

Figure 4
Interaction network of the dynamic immune response to *Candida albicans* in human full-blood samples.

however, the difference of these phagocytes with regard to the *quantitative* impact on the phagocytosis of *C. albicans* has to be determined from fitting the reaction rates to the experimental time series data.

In summary, the implemented computational framework for the dynamic modelling of interaction networks underlying immune responses represents a valuable systems biology tool to advance the quantification of dynamic infection models.

5 Infectious damage adaptation of mitochondrial dynamics

Group Leader: Marc Thilo Figge

Mitochondria are organelles that play a central role as 'cellular power plants'. The cellular organisation of these organelles involves a dynamic spatial network where mitochondria constantly undergo fusion and fission associated with the mixing of their molecular content. Together with the processes of mitophagy and biogenesis of mitochondrial mass, this results into a cellular surveillance system for maintaining their bioenergetic quality. The accumulation of molecular damage in mitochondria is associated with various human disorders and with aging. However, how these processes affect aging and how they can be reconciled with existing aging theories is just beginning to be considered. Several theories aiming to explain aging in eukaryotes ascribe a crucial role to mitochondria. One hypothesis dominating the last decades, known as the 'mitochondrial free radical theory of aging' (MFRT), states that reactive oxygen species (ROS), predominantly generated within the mitochondrial electron transport chain (ETC), cause molecular damage in a cumulative manner. Later refinements of this theory suggested a vicious cycle to occur since mitochondrial DNA (mtDNA) encoding essential

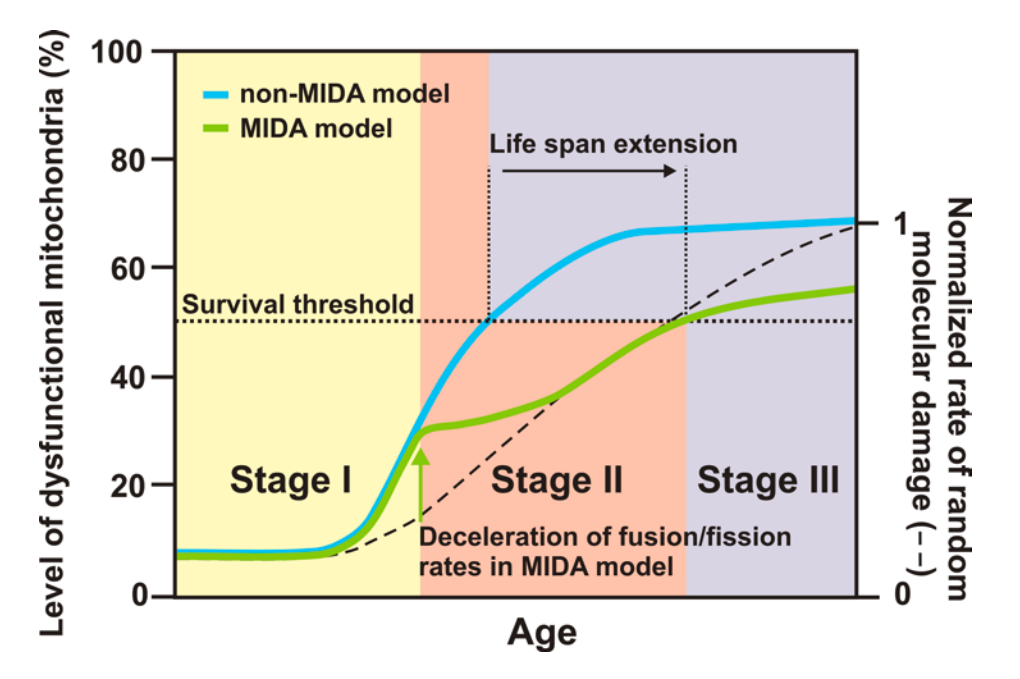

Figure 5
Stages of the 'mitochondrial infectious damage adaptation' (MIDA) model during cellular aging.

subunits of the ETC are damaged by ROS. Although the existence of a vicious cycle is unclear, it is undisputed that oxidative stress contributes to and mitochondrial dysfunction is involved in aging processes.

Recently, mitochondrial quality control was linked by several studies to the astonishing dynamic organisation of the mitochondrial network and to the selective removal of dysfunctional mitochondria by mitophagy. Mitochondria constantly undergo fusion and fission events and impairment of this dynamic behavior is linked to a range of neurodegenerative diseases and aging. Mitochondrial fusion was proposed as a mechanism primarily mediating content mixing and by that allowing inter-mitochondrial complementation compensating for missing or dysfunctional gene products of individual mitochondria. The mitochondrial life cycle thus appears to represent an efficient mechanism ensuring the molecular quality of the cellular ensemble of mitochondria. However, there are a number of observations that appear counterintuitive considering such a mitochondrial quality control system. For example, in a cellular model of aging the rate of both fusion and fission events was observed to become reduced by more than 80% in aged cells compared to young cells. As aging is well known to be associated with the accumulation of molecular damage, an increase in mitochondrial dynamics would be expected to better cope with this situation. Moreover, it was shown that ablation of mitochondrial fission extends the life span of the two fungal species *Podospora anserina* and *Saccharomyces cerevisiae*. These experiments reveal that a reduced fission rate retards aging without impairing fitness and fertility of the mutants which also appears incompatible with a fission-dependent mitochondrial quality control mechanism.

Taken together, apparent discrepancies suggest that mitochondrial quality control and its

age-dependent regulation might be more complex than initially assumed. This prompted us to apply a systems biology approach which aims to integrate current views in mitochondrial biology during aging into a probabilistic model that is described by the Master Equation approach. The *in silico* model considers alterations of mitochondrial dynamics as an adaption to the accumulation of molecular damage in aging cells that were studied by computer simulations for different scenarios of aging in order to reconcile the counterintuitive experimental observations. In particular, we investigated the possibility that mitochondrial dynamics itself could be harmful, for example in situations when molecular damage has accumulated to some degree and further content mixing would lead to an infection-like phenomenon causing molecular damage to spread across the entire mitochondrial population.

To study the impact of aging processes on mitochondrial quality control, we considered two different types of molecular damage which we termed: (i) 'random molecular damage' generated by ROS or by other processes impairing the molecular function of constituents (e.g. lipids, proteins, DNA) of mitochondria, and (ii) 'infectious molecular damage' occurring during the molecular exchange between mitochondria via fusion-fission events. Random molecular damage is well documented to occur and a vast literature demonstrates that it is implicated in the aging process. The nature of 'infectious molecular damage' is less understood but is also documented to exist as, e.g. a partial dissipation of the mitochondrial membrane potential was observed to occur upon fission of mammalian mitochondria. On top, this may be accompanied by loss of mitochondrial ion homeostasis, or the spreading of a dominant-negative factor. These two types of molecular damage have in common that they give rise to a functional impairment of mitochondria which is well reflected by our computer simulations and leads to the 'mitochondrial infectious damage adaptation' (MIDA) model of aging.

The MIDA model of aging considers the possibility of inter-mitochondrial infection by molecular damage and may be divided into three different stages during lifetime. At first, molecular damage occurs randomly by which the functional quality of an initially relatively small fraction of mitochondria becomes reduced. Nevertheless, even a small fraction of randomly damaged mitochondria can have severe consequences when this damage is propagated by cycles of fusion and fission. However, this damage is not only propagated but even enhanced as fusion and fission themselves cause another type of damage, which we termed 'infectious molecular damage'. We suggest that mitochondria that have been randomly damaged may be held responsible for infectious molecular damage in subsequent fusion-fission events. Once the overall molecular damage gets established in the mitochondrial network the associated loss in functional quality of mitochondria and in the function of the whole cell is assumed to be counteracted by a systemic response. This marks the second stage of the mitochondrial infection scenario, where the rate of fusion-fission events is adapting to lower values in order to limit spreading of molecular damage in the cellular system. How this is regulated mechanistically is unclear, but it is consistent with a study reporting that during cellular aging fusion and fission rates are strongly reduced. Rather than viewing this reduction as a main cause of aging, this may actually be a systemic response to prolong cellular functioning. Finally, the third stage is characterised by increased ROS production in aging cells as observed in numerous systems during aging. The adapted system with decreased fusion-fission rate, however, is now in a more vulnerable state less capable to cope with this situation. While the impact of random molecular damage may be significantly delayed by the adaptation of the system in stage two, this kind of damage will ultimately gain the upper hand, enforce the systems transition into stage three, and finally cause cell death. The MIDA model reconciles a number of counterintuitive observations obtained dur-

ing the last decade including infection-like processes of molecular damage spread, the reduction of fusion and fission rates during cellular aging, and observed life span extension for reduced mitochondrial fission. Our *in silico* model confirms the current view that cycles of fusion and fission and mitophagy represent a reliable and robust mechanism of maintaining functional mitochondria. In fact, the computer simulations show that this mechanism does not only maintain a functional and dynamic network of mitochondria but is also capable of driving the system into this state. Moreover, our study provides some unexpected and conceptually important findings regarding the role of mitochondrial dynamics during aging. Interestingly, the MIDA model suggests that a reduction in mitochondrial dynamics rather than being merely a sign or even cause of aging, may actually reflect a systemic adaptation to prolong organismic life span. Thus, we propose that a decrease of the fusion-fission rate as a sign of growing old may actually be viewed in a positive way, namely as the adaptation of this complex biological system to counteract the fate of dying young.

The predictions made by the MIDA model, e.g. about the impact of the deceleration of mitochondrial dynamics of organismal aging in general, will help to design experiments for testing it, to initiate refinements/extensions of the model, and in the long run to better understand whether aging is caused by an infection-like phenomenon.

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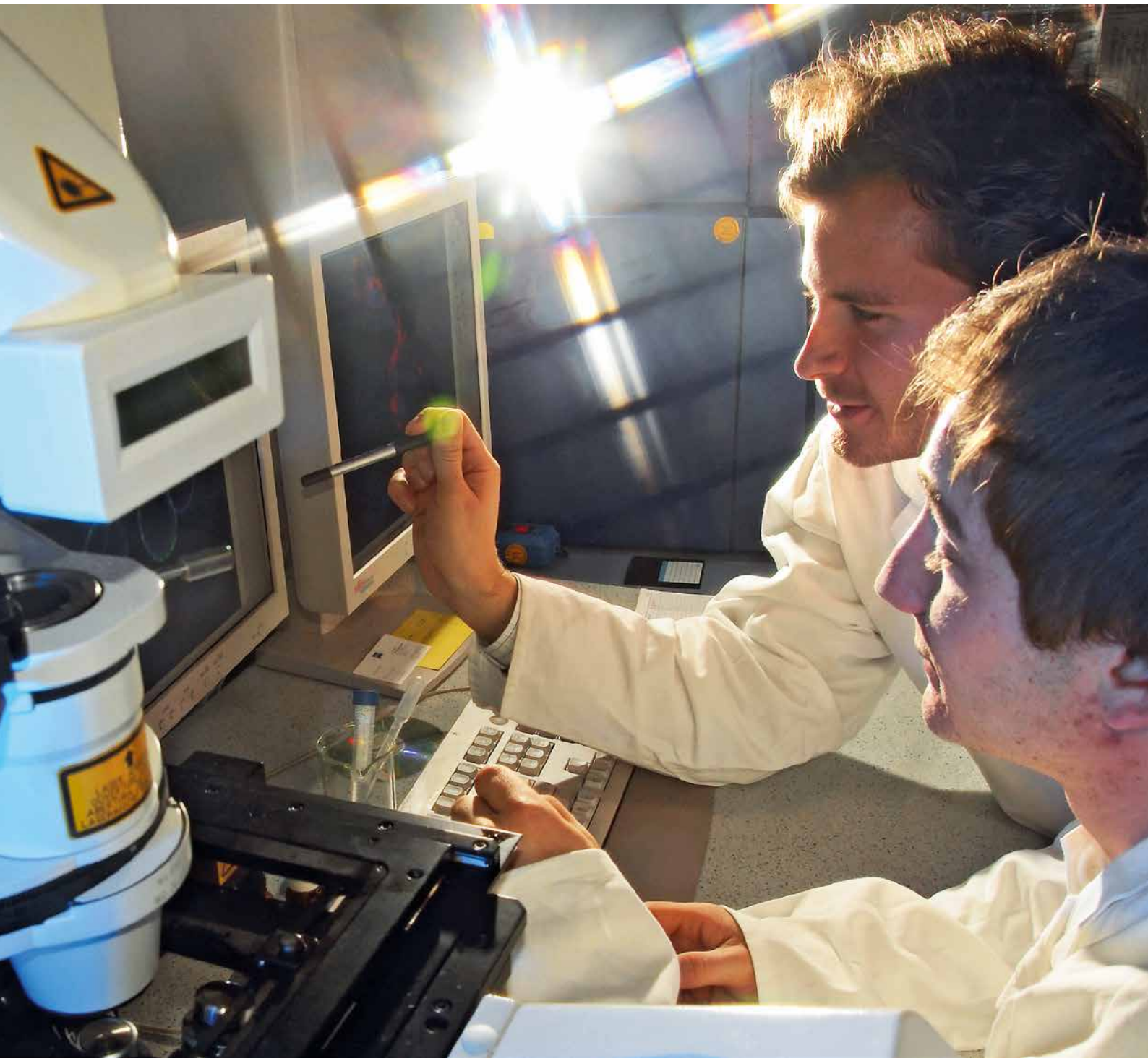
Selected publications

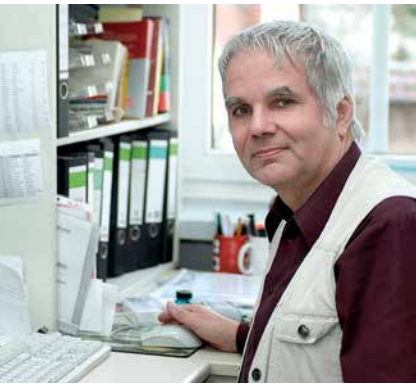
Mech F, Thywißen A, Guthke R, Brakhage AA, **Figge MT** (2011) Automated image analysis of the host-pathogen interaction between phagocytes and *Aspergillus fumigatus*. *PLoS ONE* 6, e19591.

Garin A, Meyer-Hermann M, Contie M, **Figge MT**, Buatois V, Gunzer M, Toellner KM, Elson G, Kosco-Vilbois MH. (2010) Toll-Like Receptor 4 signaling by FDC is pivotal for delivering the consequences of germinal center responses. *Immunity* 33, 84-95.



Research Group
Systems Biology and Bioinformatics

Research Group Systems Biology and Bioinformatics



The Research Group Systems Biology and Bioinformatics (SBI) is devoted to computational analysis of experimental data received from the microbiological and biomedical research. This analysis aims to establish mathematical models and model-based predictions of experimentally testable hypotheses. The integrative data analysis that is applied to identify the structure and parameters of the models includes experimentally measured data as well as prior knowledge extracted from biomolecular databases and text mining in research papers.

The research was done within collaborative networks, such as the graduate schools JSMC and ILRS as well as the DFG-Priority Programme SPP1160 'Colonisation and Infection by Human-Pathogenic Fungi'. The integrative analysis focuses on temporally resolved high-throughput data, in particular transcriptome and proteome data and the reconstruction (so-called inference or reverse engineering) of network models of gene regulation and signalling.

Bioinformatic methods are applied mainly to infection research. Large scale genome-wide network models as well as small scale models that are focused on certain questions are inferred for fungi, in particular *Aspergillus fumigatus* und *Candida albicans*. Furthermore, the integrative analysis is applied in order to understand and quantify the processes of

INTRODUCTION | EINLEITUNG

Head:
Prof. Dr. Reinhard Guthke

Die Forschungsgruppe Systembiologie und Bioinformatik (SBI) befasst sich mit der Analyse von experimentellen Daten aus der mikrobiologischen und biomedizinischen Forschung, um über mathematische Modelle in Verbindung mit Vorwissen, das aus Datenbanken und der Fachliteratur extrahiert wird, neue molekularbiologische Zusammenhänge vorherzusagen.

Die Forschungsgruppe arbeitet an einer „Systembiologie von Pilz-Infektionen" – im Rahmen der Graduiertenschulen JSMC und ILRS sowie bis 2010 im Schwerpunktprogramm SPP1160 der Deutschen Forschungsgemeinschaft. Dabei arbeitet die SBI überwiegend an der Analyse zeitaufgelöster Transkriptom- und Proteomdaten. Schwerpunkte der Systembiologie- und Bioinformatik-Forschung sind die integrierte Analyse von Hochdurchsatz-Daten („Omics"-Daten) und die Rekonstruktion (Inferenz) von Netzwerkmodellen der Genregulation und Signaltransduktion. Diese bioinformatischen Methoden werden hauptsächlich für die Infektionsforschung angewandt, indem fokussierte oder auch genomweite Modelle der Genregulation von human-pathogenen Pilzen wie *Aspergillus fumigatus* und *Candida albicans* entwickelt werden. Ferner sind diese Arbeiten auf *A. nidulans* gerichtet, einen Modellorganismus für Sekundärmetabolitbiosynthesen. Die Forschungsgruppe Systembiologie und Bioinformatik führte derartige bioinformatische Analysen von Antibiotika-Fermentationen auch im Rahmen von Industriekooperationen durch. Im Hinblick auf künftige Forschungen zur Wirt-Pathogen-Interaktion und eingebunden in lokale und überregionale Forschungsverbünde werden

fungal biosynthesis of antibiotics and other secondary products, such as Penicillin formed by *A. nidulans* and *Penicillium chrysogenum*.

Due to the fact that infection research considers not only the pathogen but also the host and, finally, the host-pathogen interaction, system biological studies are also devoted to the high throughput data analysis and gene regulatory network inference in human and animal cell cultures, animal models, and humans. Gene expression monitoring of the immune response after infection, in autoimmune diseases, during ageing as well as in healthy and diseased hepatocytes and other liver cells were studied and mathematically modeled. To develop and evaluate the strength of bioinformatic tools for network modelling, we included also studies on the best studied model organism *Escherichia coli* exploiting the huge amount of experimental data and well-structured prior knowledge that is public and online available.

Transcriptome data, gained in the past mainly using microarrays, nowadays increasingly includes RNA-seq data based on Next Generation Sequencing techniques, which will be probably become the standard technology. The RNA-seq technique opens a way of monitoring gene expression profiles from pathogens and hosts simultaneously and, therefore, to genome-wide studies of the host-pathogen interactions.

die Methoden der Transkriptomanalyse und Netzwerkinferenz auch auf humanmedizinische Prozesse der Immunantwort auf Infektionen, der Autoimmunität, des Alterns und der Genregulation von Hepatozyten und anderen Leberzellen angewandt. Zum Zwecke der Entwicklung neuer bioinformatischer Methoden wurden auch Forschungen am Modellmikroorganismus *Escherichia coli* durchgeführt, weil hier eine Fülle von experimentellen Daten und umfassendes Vorwissen verfügbar ist.

Die Analyse von Transkriptomdaten, die in der Vergangenheit vorwiegend auf Basis von Mikroarrays gewonnen wurden, bezieht zunehmend auch Daten ein, die mittels der Hochdurchsatz-Sequenzierung (RNA-Seq) gewonnen werden. Ein weiterer Schwerpunkt betrifft die Sequenzanalyse von Genomen und Proteinen. Dabei werden Transkriptionsfaktoren (TF) und Transkriptionsfaktorbindestellen (TFBS) für die Erforschung genregulatorischer Netzwerke vorhergesagt. Zudem werden mittels *Genome Mining* neue Gencluster für die Biosynthese von Sekundärmetaboliten vorhergesagt als Voraussetzung für die Aktivierung stiller Gencluster. Es wird von der Forschungsgruppe Systembiologie und Bioinformatik eine Datenbank „FunTF“ für TF und TFBS von pathogenen Pilzen aufgebaut.

The research of the Systems Biology and Bioinformatics Research Group is also devoted to sequence analysis of genomes and proteins. One of the directions is the prediction of transcription factors (TFs) and TF binding sites (TFBSs) as constituting elements of gene regulatory networks. To this aim, SBI developed novel bioinformatic tools and establishes a database for TFs and TFBSs in fungal genomes. The FunTF database is oriented mainly on human pathogenic fungi, such as *C. albicans*, and filamentous fungi, e.g., Aspergilli. Furthermore, the genome mining and analysis of secondary metabolite synthesis gene clusters carried out in our group gives hints to discovering novel natural products.

1 Systems biology of fungal infections

Group Leaders: Jörg Linde, Fabian Horn, Sebastian Müller, Steffen Priebe, Reinhard Guthke

The Research Group's work in the field of systems biology of fungal infections aims at describing and analysing the confrontation of the host with fungal pathogens, in particular *A. fumigatus* and *C. albicans*. It intends to understand and to mathematically model the interaction of the host, in particular the immune system of humans or animals, with components of pathogens. This comprises integrative analysis of genome-wide data from both the host and the pathogen. In perspective, the host-pathogen interaction should be described by a combination of spatio-temporal models with interacting molecular networks of the host and the pathogen. The SBI group focuses on genome-wide data analysis to infer network models while the newly established Research Group Applied Systems Biology is devoted to image-based modelling (see that section). The aim of both activities is to unravel the main mechanisms of pathogenicity by *in silico* methods, to identify diagnostic biomarkers and potential drug targets, and to explore novel strategies for personalised therapy by computer simulations.

Management and integrated analysis of high-throughput data

Since 2007, the group SBI has established the data warehouse "Omnifung" that is used to manage genome-wide experimental data, in particular those that monitor the genome, transcriptome and proteome from both pathogenic fungi and host. This data warehouse comprises experiment annotation and software tools for storage, visualisation, interlinking of raw data and its pre-processing, such as quality control, background correction, normalisation and imputing of missing data. Standard Operating Procedures for the integrative data analysis have been established. The web-based tool "FungiFun" for functional categorisation of fungal genes and proteins was developed and published in 2010. The tool "Ondex" for visualisation and integrated analysis of different types of 'omics' data was extended, its performance being substantially improved by Fabian Horn and he has applied the tool to data from *A. nidulans*. The data warehouse and software tools are used to assist collaborative research projects within the Hans Knöll Institute as well as within national and international collaborative networks.

Systems biology of *Aspergillus fumigatus* infections

The Research Group Systems Biology and Bioinformatics has done the first steps towards systems biology of *A. fumigatus* infections. Transcriptome and proteome data from *A. fumigatus* monitoring heat shock response were analysed and related to each other by different kinds of mathematical models and algorithms, in particular by so-called 'Coinertia' analysis and Ordinary Differential Equation (ODE)-based dynamical models. Differentially expressed genes as well as differentially regulated proteins were functionally categorised by Gene Set Enrichment Analysis (GSEA). By collaboration with Hubertus Haas (Medical University Innsbruck), Jörg Linde from the Research Group reconstructed gene regulatory networks that mathematically model the regulation of iron homoeostasis in *A. fumigatus* (Figure 1). The network inference was performed by analysis of gene expression profiles as well as soft integration of already known regulatory interactions. The inferred model predicts new interactions between transcription factors and target genes, in particular the activation

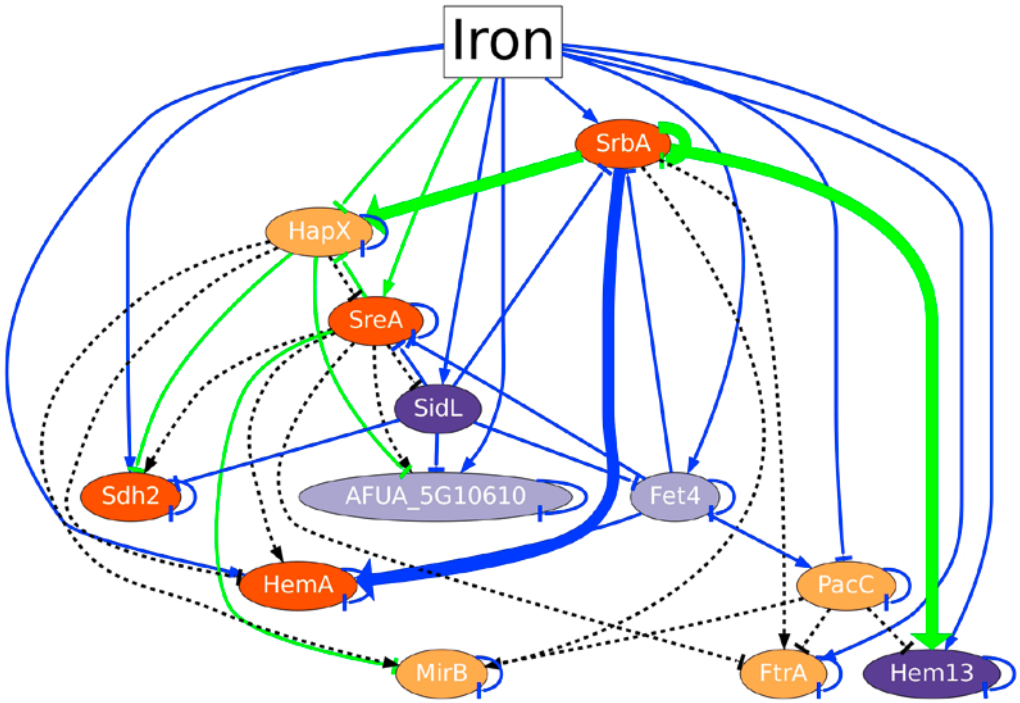


Figure 1
Gene regulatory network predicted for *Aspergillus fumigatus* under the condition of iron limitation (modified from Linde *et al., BMC Sys Biol* 2012).

of the gene expression of *hapX* by the transcriptional regulator SrbA. This interaction was experimentally verified by Northern blots by the collaboration partners. Next, the computational model of the regulation of iron homeostasis in *A. fumigatus* was refined by integration of further, more recent, just published experimental findings. Thus, the refined model includes three SrbA target genes *hapX*, *hemA* and *srbA* (Figure 1). These predicted regulatory links were further investigated by motif search within the regulatory regions of the target genes to identify potential DNA-binding sites for SrbA. Finally, high-affinity binding of SrbA to the promoters of the three predicted target genes was verified by *in vitro* experiments by Peter Hortschansky (Dept. Molecular and Applied Microbiology). This collaborative work demonstrates that a closed systems biology circle comprising dry lab work for data analysis, mathematical modelling and model-based experiment design as well as wet lab work for experimental data generation and testing of hypotheses is able to reveal novel gene regulatory interactions (see Figure 2).

Currently, in collaborative work with Vito Valiante (Dept. Molecular and Applied Microbiology) the transcriptome of the wild type and the mpkA mutant of *A. fumigatus* is analysed by Sebastian Müller from the Research Group Systems Biology and Bioinformatics using paired-end RNA-seq data from Illumina Genome Analyzer IIx and from four different microarray platforms in comparison with 2D DIGE proteome data. By this study, we identified a substantial number of novel transcripts, single nucleotide polymorphisms (SNPs), and new splice junctions predicting widespread alternative splicing events in *A. fumigatus*. Known and unknown gene clusters, which are involved in secondary metabolite production, were identified to be differentially regulated in the mutant versus wild type.

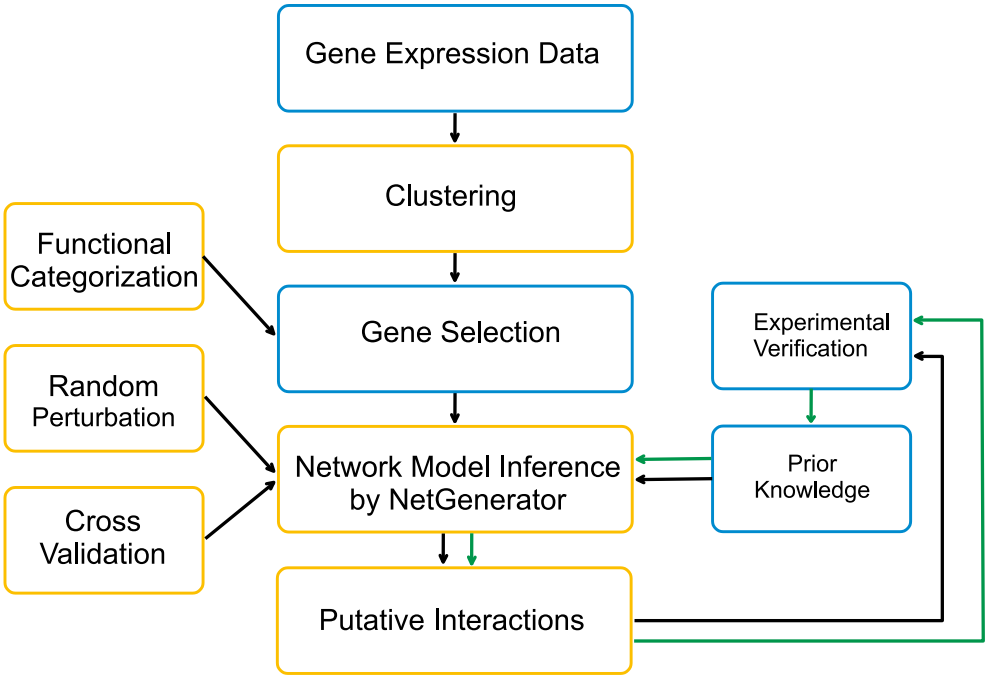


Figure 2
Cyclic workflow for inference of a gene regulatory network model (Figure 1) applied to the human pathogenic fungus *Aspergillus fumigatus* (modified from Linde *et al., BMC Sys Biol* 2012).

The automated image analysis of the host-pathogen interaction between phagocytes and *A. fumigatus* was performed within the Research Group by Franziska Mech who continued this work in the 2011 newly founded Research Group of Applied Systems Biology headed by Marc Thilo Figge. First results were published in 2011.

Systems biology of *Candida albicans* infections

To model gene regulatory networks of the fungus *C. albicans*, the Research Group Systems Biology and Bioinformatics applied both a large scale genome-wide and a small scale focused approach. By the latter one, Jörg Linde investigated the regulation of iron uptake and, thus, the adaptation process of the fungus to conditions of very low iron availability which is an important requirement during the infection process. The 19-gene-network model represents the regulation of 15 target genes by the four transcription factors Rim101, Hap3, Sef1, and Tup1 under the condition of iron limitation.

To investigate central genes, so-called hubs, Robert Altwasser developed large scale genome-wide network models of gene regulation in *C. albicans*. Gene expression data of this fungus under 198 experimental conditions such as drug treatment were analysed together with several sources of prior knowledge about transcription factors and their binding sites and about protein-protein interactions (obtained from databases, such as BIND and TRANSFAC). Since the prior knowledge provided in online databases for *C. albicans* is insufficient, prior knowledge derived from other fungal species had to be included. To assess and minimise the bias caused by usage of knowledge from other species, the inferred networks have been evaluated by comparison of the network edges with the knowledge from a *C. albicans*-specific 'gold standard'. The 'gold

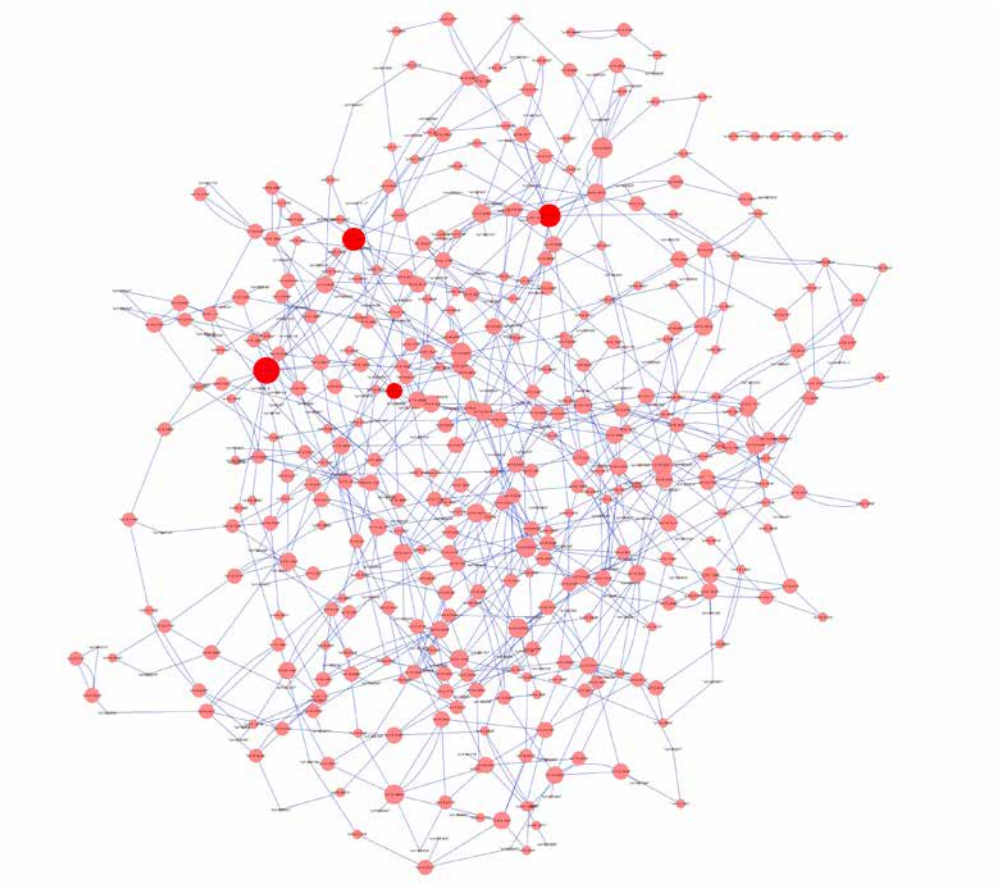


Figure 3
Result of the inference of a genome-wide gene network: Sub-network of the 503 'gold standard' genes, for which gene – gene interactions were extracted by text mining in 9000 research papers on *Candida albicans*.

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standard' comprising 503 genes with 971 interactions has been extracted from 9000 full text research papers by the use of text mining techniques (collaboration with the Language and Information Engineering Lab of the Friedrich Schiller University Jena). Figure 3 shows the sub-network for the 503 genes that are a part of the 'gold standard'. We found 126 hubs with an out degree of at least seven. A total of 16 hubs were found to be influenced by known antimycotica such as amphotericin B, caspofungin or the azole group. A sub-network of GAL-genes, the gene regulation of which is well-known in *Saccharomyces cerevisiae* was studied in more detail. The genome-wide model predicts a regulation cascade from GAL10 via GAL1 to GAL7, where only the second link is already known and, therefore, is part of the gold standard. The first link is already known in *S. cerevisiae* and was predicted for *C. albicans* by our network model.

In principal, a molecular model of infection has to comprise interacting molecular networks of both the host and the pathogen. Gene expression analysis by next generation sequencing offers the possibility to monitor the transcriptomic behaviour of host and pathogen simultaneously and, thus, provide data to infer the interaction network of both. First steps in such integrated systems analysis were done in collaboration with Karl Kuchler and coworkers (Medical University of Vienna).

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 several directions, such as transcription regulation modelling and genome mining/comparative

genomics of secondary metabolite genes and gene clusters.

Transcription regulation modelling

Collecting reliable sets of pre-existing data, which serve as the basis for the modelling, is crucial for the quality of the future predictions. Although the research in the field of transcription regulation in fungi is very active, there has been no database on fungal transcription factors so far. Because of that, we created a database on fungal transcription factors (FunTF). In our new FunTF database we collected experimentally verified TFs and their binding sites reported in literature. The database is manually curated and currently contains information about more than 200 TFs from 24 fungal species, 130 binding sites, and 178 regulated genes. As additional features, FunTF provides possibilities to find predicted orthologs of a given TF in several fungal species (these sets are predefined with the help of the Inparanoid tool) and to visualise known interactions of TFs and regulated genes.

To make immediate use of the collected binding sites, we provide the user with a tool called SiTaR (Site Tracking and Recognition) for TFBS prediction. Prediction of transcription factor binding sites (TFBSs) is crucial for promoter modelling and network inference. Quality of the predictions is spoiled by numerous false positives, which persist as the main problem for all presently available TFBS search methods. We suggested a novel approach, which is an alternative to widely used position weight matrices (PWMs) and Hidden Markov Models. Each motif of the input set is used as a search template to scan a query sequence. Found motifs are assigned scores depending on the non-randomness of the motif's occurrence, the number of matching searching motifs, and the number of mismatches. The non-randomness is estimated by comparison of observed numbers of matching motifs with those predicted to occur by chance. The latter can be calculated given the base compositions of the motif and the query sequence. The method does not require preliminary alignment of the input motifs, hence avoiding uncertainties introduced by the alignment procedure. In comparison with PWM-based tools, our method demonstrates higher precision by the same sensitivity and specificity. It also tends to outperform methods combining pattern and PWM search. Most important, it allows reducing the number of false positive predictions significantly. The method was published in 2011 and the tool is available at http://sbi.hki-jena.de/sitar/index.php.

Another tool for promoter analysis developed in our group deals with the next step of the TFBS prediction, namely looking for meaningful combinations of the binding sites. To interact, TFs should bind sites which are located at certain distances. These distances are not random. This feature distinguishes them from the rest of the predicted sites, the distances between which are randomly distributed. The new tool utilises a method of distance distributions of TFBS pairs previously developed in our group. We model the random distribution of distances and compare it with the distribution observed in the query sequences. Comparison of the profiles allows filtering out the 'noise' and retaining the potentially functional combinations. This approach has proved its usefulness as a filtering technique for the selection of TFBS pairs for promoter modelling and is now implemented in R as a tool called DistanceScan. The tool was published in 2010 and is available at https://www.omnifung.hki-jena.de/Rpad/Distance_Scan/index.htm.

Taken together, our efforts lead to the possibility to apply a workflow based on the collected reliable data sets and provided high quality tools. The workflow allows to obtain predictions for transcription regulation networks and various details of particular regulatory pathways.

Our group works in tight contact with the experimental labs of the HKI. Phylogenetic analysis of particular groups of genes, prediction of potentially secreted proteins, and

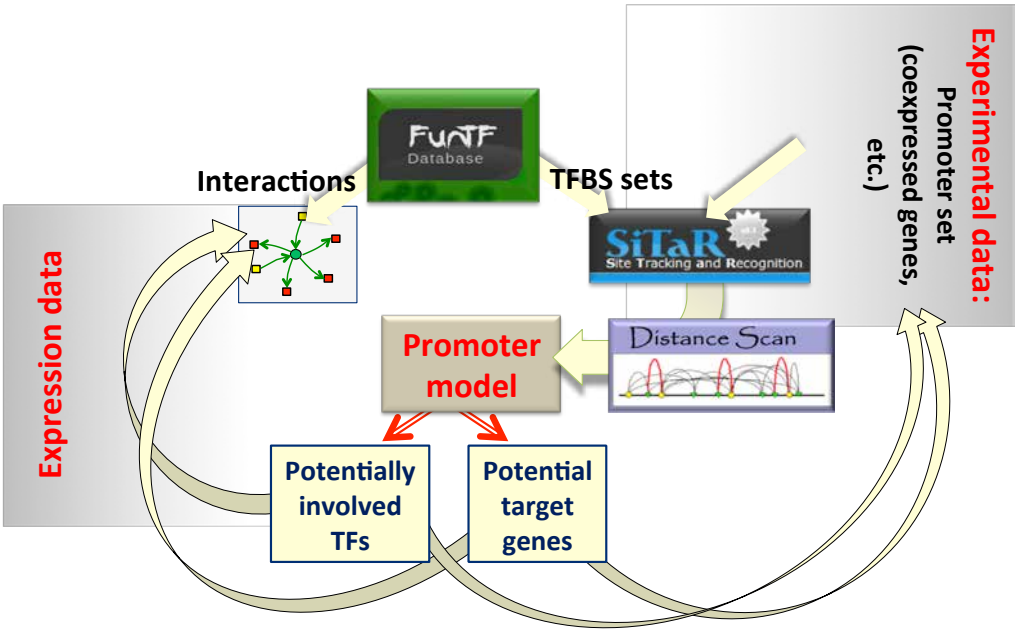

Figure 4
The workflow for transcription factor binding site prediction and inferring transcription regulation networks.

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in the first place the promoter analysis of the genes of interest are among our everyday activities. An example of such collaboration is the work of our colleagues from the Dept. Molecular and Applied Microbiology and Dept. Biomolecular Chemistry discovering a regulatory cross talk of two secondary metabolite gene clusters (Bergmann *et al.*, 2010).

Genome mining/comparative genomics of secondary metabolite genes and gene clusters

Fungi are known to produce pharmaceutically important natural products (secondary metabolites, SMs) synthesised by polyketide synthases (PKS) and nonribosomal peptide synthetases (NRPS). Analyses of sequenced fungal genomes have revealed numerous examples of 'cryptic' biosynthetic gene clusters, which can potentially be responsible for the production of the novel SMs. On the other hand, for many known SMs the corresponding biosynthetic pathways are unknown. Our work is devoted to the genome-driven detection of new complex natural products in selected fungi and bacteria.

In bioinformatic analysis, the experimentally verified data are of special value. They serve as ground for modelling and are used for the validation of predictions. Therefore, we created a database of all so far known fungal PKSs and NRPSs with biochemically characterised products. In total, the database contains 103 confirmed fungal SM genes responsible for 96 types of products in 54 different fungal species.

Based on the collected data, we were able to analyse the properties of known and predicted SM gene clusters in order to develop approaches for the clusters' prediction. The analysis of the surroundings 30 known PKSs/NRPSs (± 30 genes) revealed the prevalence of several protein families (transcription factors, transporters, transferases,

etc.) However, we can state that there is no single protein family, which could be used as a distinguishing feature of a cluster.

In order to see how and to which extend we can incorporate RNA-seq data in cluster prediction approaches, we analysed the behaviour of the known and supposed cluster genes in the RNA-seq data of *A. fumigatus*. Unfortunately, in many cases the genes beyond the clusters show the same behaviour as the cluster genes, so we cannot see the cluster boundaries in the expression profiles. Moreover, in some examples not all known cluster genes are expressed simultaneously, probably due to sequential induction of later pathway genes, or to the non-synchronised activation of the cluster genes. In any case, we can state that the expression profiles can be used only as supporting data, but the cluster prediction cannot be based solely on this evidence.

As it has been mentioned above, evolution of the SM genes is one of the directions of our research. One of the most intriguing questions is the evolution of PKS/NRPS hybrids, the main question being the moment of the fusion of the PKS and NRPS moieties. We considered 65 PKS/NRPS hybrid sequences from 28 fungal species, including 11 hybrids with known functions/products. The phylogenetic trees were made for the whole hybrids, separately for the PKS and NRPS moieties, and for several selected domains (ketoacyl-synthase, acyltransferase, adenylation, and methyltransferase domains). The trees were then compared by creating pairwise tanglegrams. The results suggest that the PKS and NRPS parts share the history and most likely have merged before speciation.

Annotation, genome mining, and phylogenetic analysis of SM genes/gene clusters and transcription factors in dermatophyte species was a part of the *Arthroderma benhamiae* genome project, the first genome project of a dermatophyte in Europe. The consortium was led by the HKI in cooperation with the Leibniz Institute for Age Research - Fritz Lipmann Institute (FLI), and the Friedrich Schiller University Jena. The results were published in 2011.

3 Gene regulatory network inference

Project Leaders: Michael Weber, Sebastian Vlaic, Wolfgang Schmidt-Heck, Reinhard Guthke

The function and dynamics of molecular entities, e.g., genes, proteins, metabolites, that constitute the cellular behaviour of living systems are usually represented by gene-regulatory networks, signalling networks, protein-protein interaction networks, and metabolic networks. The confrontation between pathogen and host can be viewed as two interacting molecular networks. Thus, the reconstruction of these networks is a crucial task in systems biology for natural product research as well as infection biology. Networks consist of nodes and edges that interlink the nodes and represent, e.g., interactions. The nodes that represent the aforementioned molecular entities are quantified by concentrations or activities. These variables can be described by logic values ('on', 'off', or 'present', absent', 'marginal'), by discrete values (e.g., cluster labels), fuzzy values (e.g., 'low', 'medium', 'high') or continuous values (e.g., concentrations or expression intensities). The research of the Systems Biology and Bioinformatics group has a longstanding tradition in the field of network modelling. All modelling architectures were applied. A temporal logic approach was the central idea for network modelling of the extracellular matrix formation in the human synovial membrane by Johannes Wollbold who finished his PhD thesis in 2011. An Adaptive Neuro-Fuzzy Inference System is applied by Wolfgang Schmidt-Heck to model the Hedgehog pathway in murine hepatocytes – a project embedded within the German Systems Biology initiative on Virtual Liver.

However, most scientific activities are directed on modelling of gene regulatory networks using continuous variables where

the network dynamics and interactions are formulated by Ordinary Differential Equations (ODE) or Difference Equations. The latter ones as well as information theory based approaches are generally used for medium and large scale modelling, in particular for modelling of genome-wide networks, while ODE-based modelling is applied to reconstruct small scale networks with the aim to solve specific questions.

Focused, small scale modelling

Small scale modelling network implies selection of a small set of genes (e.g., 5 to 20 genes), which can be based on the selection of particular features of interest. In the Research Group Systems Biology and Bioinformatics, the problem of the small set selection was approached by the following steps: First, differentially expressed genes (DEGs) are identified to generate a set of candidate genes. Next, the DEGs have to be grouped with respect to their kinetic behaviour, e.g., by fuzzy clustering of gene expression profiles, and with respect to their function by GSEA using the knowledge available in databases such as FunCat, Gene Ontology (GO), KEGG, Protein-Protein Interaction and literature databases (e.g., BIND). Finally, a small set of genes of interest for modelling is defined by selection of one or a few of representative genes from each group or cluster. Certain functional groups, e.g., genes coding for transcription factors and genes known to be involved in a process of special interest, are pre-selected. In our research, such process of interest was the regulation iron uptake and iron homeostasis in *C. albicans* and *A. fumigatus* (see chapters above: Systems biology of *C. albicans* infections and Systems biology of A. *fumigatus* infections). The network modelling for the 19 genes of *C. albicans* and 12 genes of *A. fumigatus* was performed using the ODE-based NetGenerator tool (Figure 1). To overcome the problem that the number of experimental data is insufficient to estimate all model parameters, first, sparse networks are preferred (i.e., the most of the elements of the interaction matrix are set to zero), and, second, prior knowledge is included whenever it is available. Prior knowledge about regulator - target gene interactions comes from different sources with different confidence. The relevance of prior knowledge can vary for different reasons, e.g., because the data have been obtained in different experimental set up and in different fungal species. The knowledge available specifically for *C. albicans* or *A. fumigatus* is scarce. So we had to include also data gained from cognate species (e.g., *S. cerevisiae*) even though we do not expect that all such data will be valid for the pathogenic fungus of interest. Therefore, the knowledge from different sources has been weighted by scores and used as input for the network inference algorithm NetGenerator. Using soft integration of prior knowledge, knowledge that contradicts experimental data has been skipped. Cross validation was applied to keep only such putative interactions that are robust against small variation (perturbation, disturbance) of measured data as well as against minor change of the prior knowledge.

By this focused network modelling approach that integrates both experimental data and prior knowledge using the NetGenerator algorithm, we could predict hitherto unknown regulator-target gene relations for *A. fumigatus* (see chapter Systems biology of *Aspergillus fumigatus* infections). The circle of data analysis via network inference to experimental testing of model-derived hypotheses was run twice, i.e., the network model was refined by incorporation of very new experimental findings (Figure 2). To test the hypotheses derived from the refined network model, potential transcription factor binding sites has been identified by promoter sequence analysis, that, finally, was experimentally verified by protein-DNA-binding assays designed *in silico* and performed by the in-house collaboration partner Dept. Molecular and Applied Microbiology.

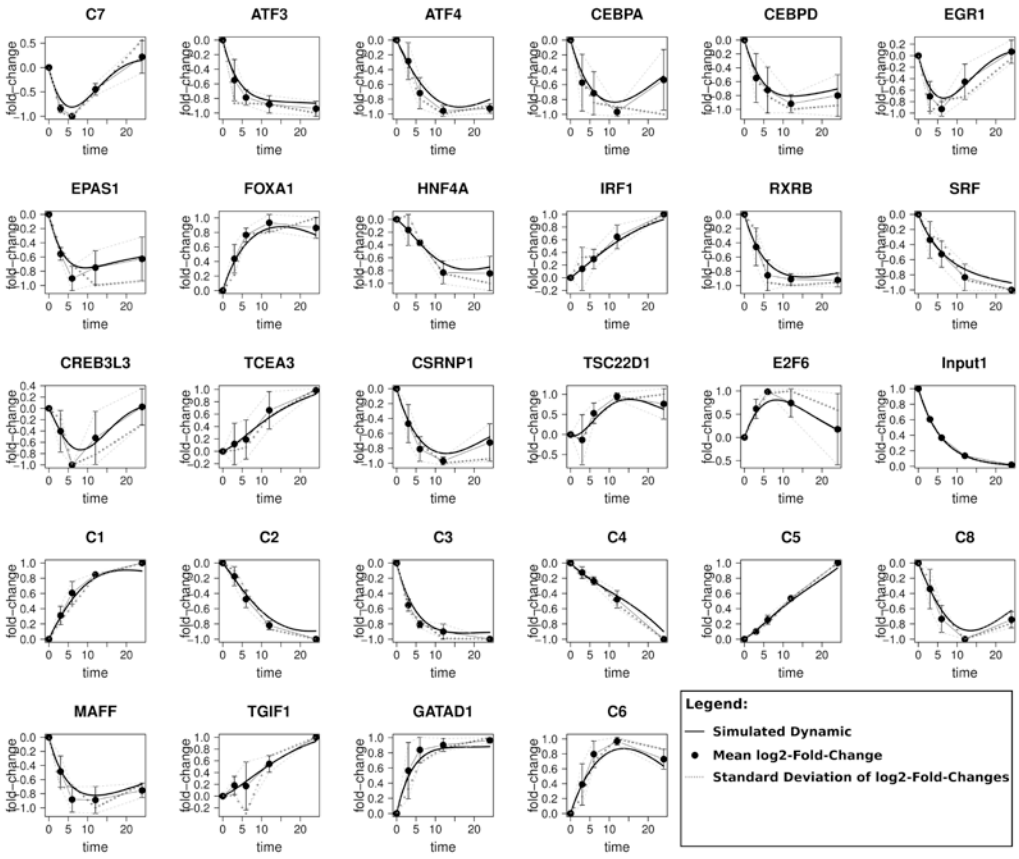

Figure 5
Expression profiles of 19 genes and 8 gene clusters (averaged kinetics) as measured by Affymetrix chips (dots) and simulated by the network model shown in Figure 6.

Large scale network modelling

Hitherto, genome-wide modelling was applied only for model organisms because it requires large collections of 'omics' data based on numerous experiments as well as comprehensive knowledge that is computationally accessible via online databases. In collaboration with Christoph Kaleta from the Friedrich Schiller University Jena, the Research Group Systems Biology and Bioinformatics applied such large scale modelling to *Escherichia coli*. A novel network model inference method, called DTI (Directed Information) that applies the Mutual Information measure together with temporal order in time series experimental data from the Many Microbes Microarray Database was used together with promoter sequence analyses to predict new transcription factor – target interactions. Regulatory knowledge extracted from the database RegulonDB was used as 'gold standard' to assess the quality of the inferred networks and to filter out novel interactions. As the result, a total of 23 new targets for the regulator PdhR (Pyruvate Dehydrogenase Repressor) was predicted which could be experimentally validated by the project partners from the University of Osnabrück and the Helmholtz Centre for Infection Research Braunschweig.

Even though the availability of experimental data and knowledge for human pathogenic fungi is not as excellent as for *E. coli*, Jörg Linde and Robert Altwasser from the Research Group published the first genome-wide network model for *C. albicans* (Figure 3). They exploited a compendium of gene expression data comprising 198 experimental conditions and integrated four sources of prior knowledge (see chapter Systems biology of *Candida albicans* infections). Here, the so-called 'Least Absolute Shrinkage and Selection Operator' (LASSO) was applied to infer a genome-wide network. Again, a 'gold standard' was necessary to assess the quality of the inferred network and, thus, to opti-

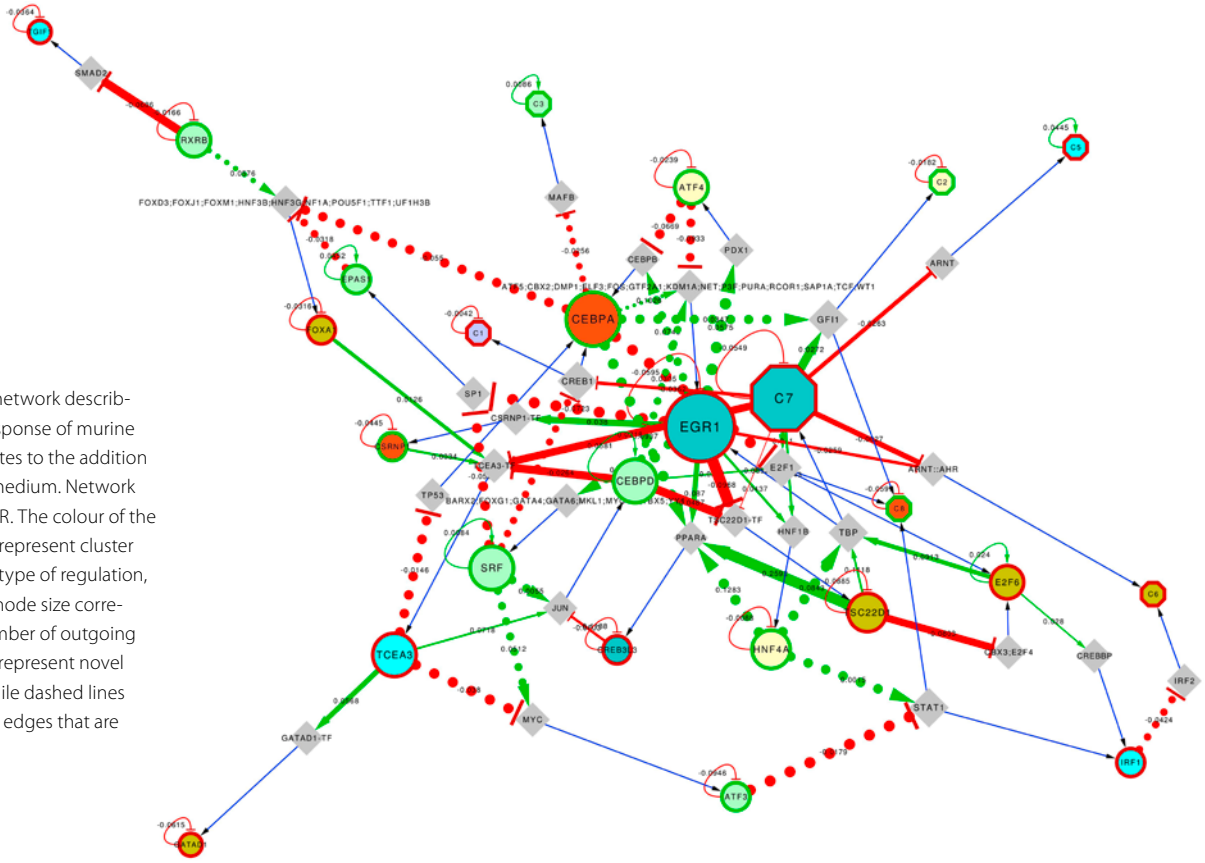

Figure 6
Gene-regulatory network describing the cellular response of murine primary hepatocytes to the addition of fresh nutrient medium. Network inferred by ExTILAR. The colour of the nodes and edges represent cluster membership and type of regulation, respectively. The node size corresponds to the number of outgoing edges. Solid lines represent novel inferred edges while dashed lines represent inferred edges that are already known.

mise two parameters for configuration of the inference algorithm. However, for pathogenic fungi there is no comprehensive online database that provides the available knowledge about gene regulatory mechanisms. Therefore, such knowledge has been extracted from literature by manual inspection of research papers to set up the novel database FunTF (see chapter Sequence analysis, genome structure and genome mining in fungi) and also by text mining (in collaboration with the Friedrich Schiller University Jena).

Medium scale network modelling

Small scale network inference is ODE-based. The tool NetGenerator for small scale network inference provides model-simulated kinetics of gene expression profiles as output. This is helpful to visualise the quality of model fit to experimental data. However, such valuable output is not available when using information theory-based or LASSO-based modelling approach for large scale modelling. To bridge over these both methods, Sebastian Vlaic from the Research Group Systems Biology and Bioinformatics developed an novel inference method, called ExTILAR (Extended transcription factor binding sites integrating least angle regression algorithm). Figure 5 shows gene expression profiles as measured by microarrays and simulated using the network model. The network model has been inferred by ExTILAR from microarray data monitoring the response of murine hepatocytes to nutrient change (Figure 6). To validate the inferred edges of the network and to keep only robust results, the network inference is repeated with perturbed data that are calculated by adding noise to the measured gene expression data.

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Project: Transcriptome data analysis and modeling of *Aspergillus fumigatus* – phagocytes interaction
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Deutsche Forschungsgemeinschaft
SPP1160 Human-pathogenic Fungi
Project: 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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Research Group Fungal Septomics

Research Group Fungal Septomics

Sepsis develops from an uncontrolled immune reaction to an infection, which affects the whole organism. Sepsis severely compromises the progress of medicine in many fields and consumes a major part of health care resources. The mortality of severe sepsis is over 50 % and has not changed much over the last decades. For these reasons, research into sepsis is urgently required to open new possibilities for improved diagnosis and treatment. With the Septomics Research Centre, the Jena expertise in sepsis research is strengthened and expanded. The fields of pathogen and host biology in sepsis will be approached complementarily and linked to clinical practice and efficient bioinformatics. Other singular possibilities at Septomics include the availability of biobanks with blood and serum specimens from patients as well as a direct link to basic science at the university and independent research institutions. Septomics is a faculty-spanning centre of the University of Jena and is scientifically interconnected with the Department of Anaesthesiology and Intensive Care Medicine (KAI) of the University Hospital Jena, the Institute for Medical Microbiology and the Leibniz Institute for Natural Products Research and Infection Biology – Hans Knöll Institute.

The spectrum of pathogens responsible for sepsis in industrialised countries has changed dramatically in the course of time, with increasing numbers of Gram-positive bacteria and fungal pathogens. In the USA, fungi of the genus *Candida* have become the

INTRODUCTION | EINLEITUNG

Head:
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Das Krankheitsbild der Sepsis entsteht durch eine unkontrollierte Immunreaktion auf eine 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 einen Großteil der Ressourcen im Gesundheitswesen. Die Sterblichkeit der schweren Sepsis beträgt über 50 % und hat sich in den letzten Jahrzehnten nicht entscheidend reduziert. Daher besteht ein hoher Bedarf an Grundlagenforschung im Bereich Sepsis, um so langfristig neue diagnostische Werkzeuge und neue therapeutische Zielstrukturen zu identifizieren. Im Zentrum für Innovationskompetenz (ZIK) Septomics soll die am Standort Jena vorhandene Expertise im Bereich Sepsisforschung verstärkt und ausgebaut werden. Die bisher weitgehend getrennten Bereiche Erreger- und Wirtsantwortforschung werden komplementär bearbeitet und mit klinischer Forschung und einer leistungsfähigen Bioinformatik verknüpft. Weitere Alleinstellungsmerkmale sind die Nutzungsmöglichkeit einmaliger 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 vom Universitätsklinikum über die Klinik für Anästhesiologie und Intensivmedizin (KAI) und dem Institut für Medizinische Mikrobiologie sowie der Biologisch-Pharmazeutischen Fakultät und dem Leibniz Institut für Naturstoff-Forschung und Infektionsbiologie – Hans-Knöll-Institut.

Das Spektrum der Krankheitserreger, die Sepsis in industrialisierten Ländern verursachen, hat sich im Laufe der Zeit drama-

third most common cause of septic infections and result therefore in considerable effects on the therapeutic outcome of the affected patients as well as on the duration of the hospital stay and costs. Besides sepsis primarily caused by *Candida spp.*, an invasive infection caused by *Candida* can also develop secondarily during a bacterial infection or a SIRS (severe inflammatory response syndrome). These secondary infections also result in deteriorating prognosis and significantly increased health care costs. Infections with other fungal pathogens, mainly invasive aspergillosis, are also 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 Septomics to put a clear focus on the pathophysiology of systemic fungal infections. Fungal Septomics analyses 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. The resulting data will lead to a better understanding of molecular mechanisms, which are based on the development of systemic infections caused by *Candida*. Furthermore they build a basis for translational projects that should lead to the development of improved diagnostic processes as well as the identification of new targets for therapeutic intervention.

tisch verändert. Dabei sind insbesondere Gram-positive Erreger und Pilze immer wichtiger geworden. In den USA sind Hefepilze der Gattung *Candida* mittlerweile die dritthäufigste 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 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 Forschungsgruppe Fungal Septomics im ZIK Septomics eine Arbeitsgruppe etabliert, die sich schwerpunktmäßig mit der Pathophysiologie systemischer Pilzinfektionen beschäftigt. Fungal Septomics untersucht molekulare Mechanismen der Pathogen – Wirt Interaktion, die zur Entwicklung von Invasivität bei *Candida albicans* führt, und Muster der Immunaktivierung im Rahmen einer Pilzsepsis. Die resultierenden Daten sollen 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.

1 Pathobiology of *Candida albicans*

Group Leader: Oliver Kurzai

Candida spp. are the most common fungal pathogens with *C. albicans* causing approximately 50% of all invasive *Candida* infections. This species is a common commensal of the human host and can be found as a harmless colonizer in the majority of the healthy population. Only rarely, *C. albicans* will penetrate the mucosal barrier of its ecological niche and can then disseminate in the bloodstream to cause invasive infection. Risk-factors for the development of invasive candidiasis include severe underlying disease, necessity for antibiotic therapy, and central venous catheters. Both in colonisation and during invasive infection, *Candida* shows an exquisite ability to adapt to conditions within the human host, including a temperature of 37°C, pH-variations between highly acidic and slightly alkaline and a high CO_2 concentration of up to 6%. The adaptation of *C. albicans* to these conditions is maintained by a complex and interacting network of regulatory cascades. Interestingly, the response of *C. albicans* to many of these conditions is closely linked to morphological plasticity of this pathogen and directly influences the transition between yeast and filamentous forms which has been shown to be crucial for virulence. Within the Fungal Septomics group, several projects address the adaptation of *C. albicans* to the environment of the host niche both during colonisation and infection. In cooperation with the group of Fritz Mühlschlegel (University of Kent at Canterbury), we have elucidated signalling pathways that contribute to adaptation of *C. albicans* to high CO_2 concentrations. Like all pathogens and many other organisms, *Candida* is able to sense and respond to changes in carbon dioxide concentrations. Within the human host, these changes can be quite dramatic with high CO_2 concentrations in the blood (5.5% CO_2) – considerably higher than for example in ambient air or at body surfaces where *C. albicans* is in continuous contact with the atmosphere (0.033% CO_2). Fluctuations in CO_2 levels are known to impact on the expression of microbial virulence determinants, and play a significant role in pathogenicity of diverse pathogens including bacteria like *Bacillus anthracis* or *Streptococcus pyogenes* and parasites like *Trichostrongylus*. Carbon dioxide sensing is known to occur in pathogenic fungal species. In *C. albicans* elevated CO_2 is a strong promoter of morphogenesis, modulating the yeast to hyphal transition as well as the white-opaque switch. In *Cryptococcus neoformans*, CO_2 has been shown to facilitate capsule biosynthesis and inhibit sexual reproduction. Insights into the signal transduction pathways that govern CO_2-sensing in *C. albicans* and *C. neoformans* have identified two prominent enzymes, carbonic anhydrase and adenylyl cyclase that play major roles in physiology and metabolism. In recent studies in cooperation with Prof. Fritz Mühlschlegel, we have been able to identify a novel CO_2 signalling pathway in *C. albicans* that acts independent of adenylyl cyclase and cyclic AMP. The key transcriptional regulator of this novel pathway is Rca1 and it is responsible for CO_2 dependent regulation of the expression of carbonic anhydrase in *C. albicans* and other fungal pathogens. ChIP-chip analyses have suggested a large Rca1 dependent regulon and analyses in other species suggest that this pathway may be conserved in several fungal species including S. *cerevisiae*. Ongoing studies address further characteristics of this new adaptive pathway. In addition to this, a strong focus is put on the link of CO_2-adaptation and other environmental signals to filamentation. In a large set of transcriptome analyses we aim to identify patterns of gene expression dur-

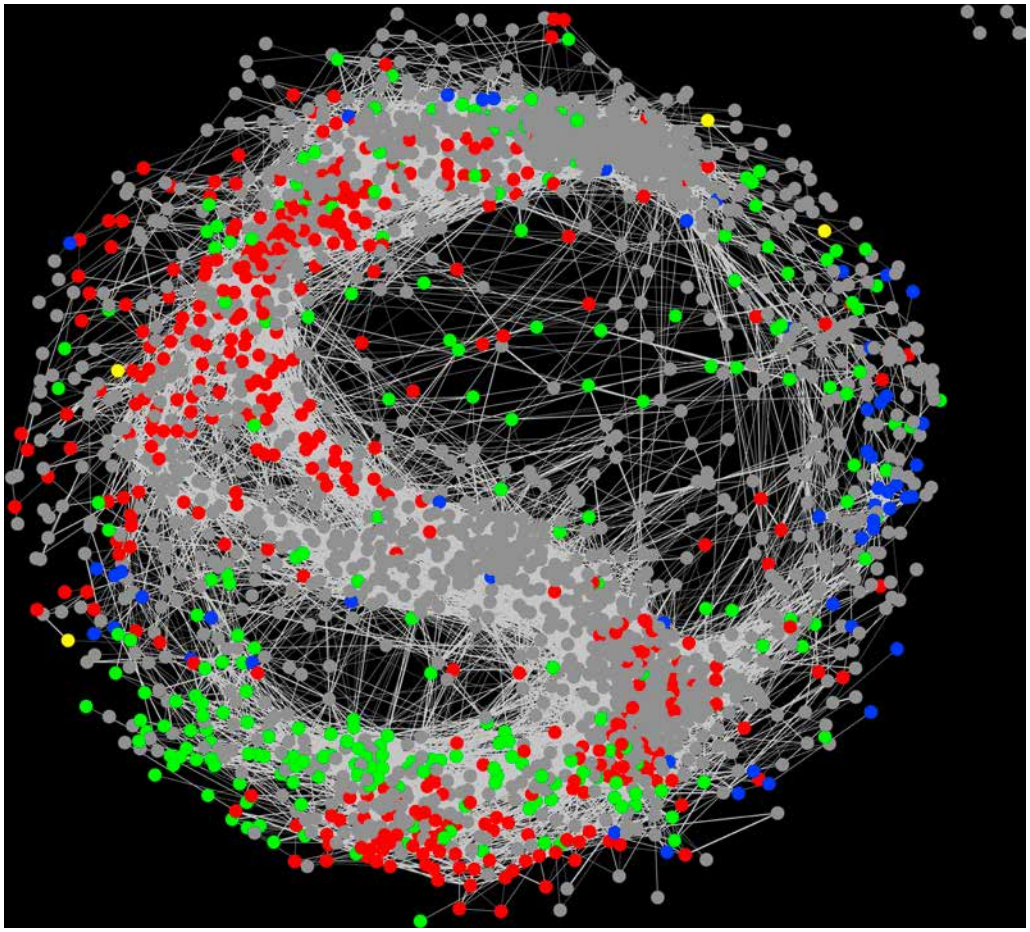

Figure 1
Transcriptional landscape of *Candida albicans* during germ tube formation.
Candida albicans was stimulated to switch from unicellular yeast cells to germ tubes by three different external stimuli: 1) shift from acidic pH4 to alkaline pH8 2) addition of 10% human serum and 3) exchange of the carbon source from glucose to N- acetylglucosamine. The fungal transcriptome was analysed 1h after stimulation and based on gene expression correlation, a network of all genes was constructed with the Cytoscape software. The model shows stimulus- specific gene expression (red: pH shift- specific genes, green: serum shift- specific genes, blue: carbon source change- specific genes) and genes which were differentially expressed in all three shift (yellow). Genes shown in grey were not differentially expressed at this specific time point.

ing this morphological switch and regulatory patterns governing this transition. For this purpose, we also employ a transwell model of intestinal barrier. In this model filamentation and active growth of *C. albicans* is linked to barrier disruption, enabling us to study the mechanisms by which *C. albicans* interferes with barrier integrity.

Immune recognition of fungal pathogens

Innate immune mechanisms play an important role in antifungal immunity and in pathological immune activation during sepsis. Neutrophilic granulocytes are the predominant type of leukocyte in the human blood and play a major role in the early containment of bacterial and fungal invasion. Targeted to a site of pathogen invasion by chemotactic mechanisms, neutrophils ingest and kill invading microorganisms. In previous work, we have been able to show that filamentous forms of *C. albicans* trigger a specific activation program in human polymorphonuclear leukocytes (PMN) which is associated with targeted motility and recruitment of additional neutrophils. We have been able to show that ERK-signalling plays an important role in the morphotype specific activation program of human PMN against *C. albicans* filaments. The role of reactive oxygen intermediates (ROI) and neutrophil granule content is currently under investigation. In addition to neutrophils, we have extended the methodological spectrum to other immune cells including natural killer (NK) cells in a close collaboration with Dr. Jürgen Löffler (University of Würzburg). The interaction of *A. fumigatus* with human primary

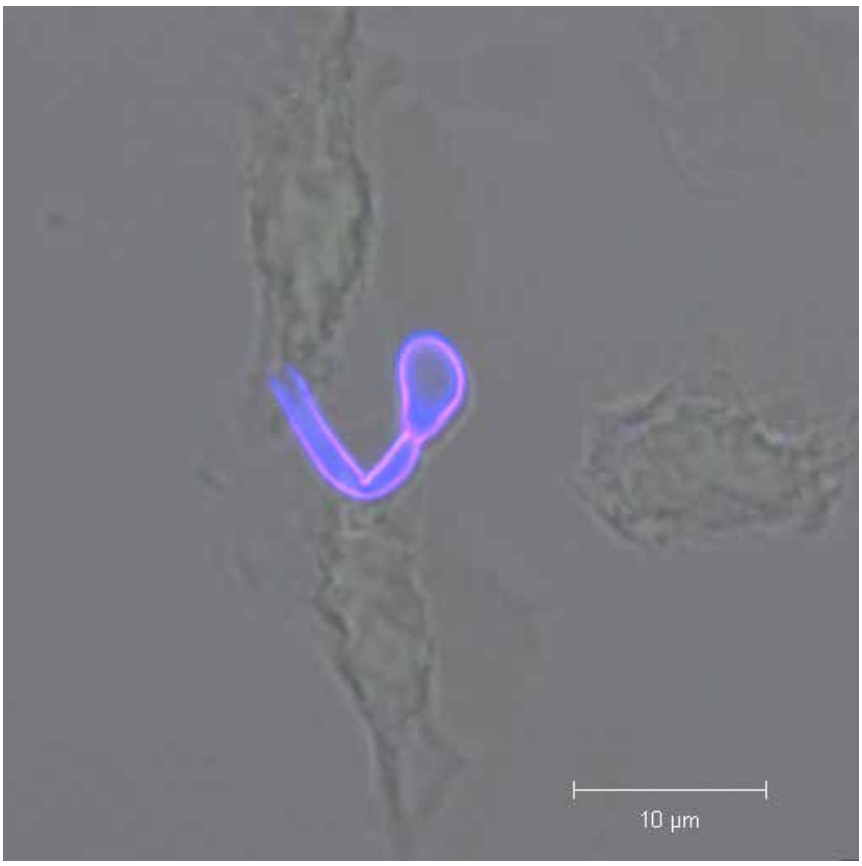

Figure 2
Human Natural Killer cells attack *Candida albicans*.
Natural Killer (NK) cells isolated from human blood and activated during short-term culture *in vitro* attack filamentous forms of the pathogenic fungus *C. albicans* (blue).

NK-cells turned out to be strongly dependent an the morphotype with only germinated morphologies but not resting conidia inducing a Th1-biased proinflammatory response, characterised by an upregulation of IFN-γ and TNF-α. The antifungal effects of NK-cells depended both on moderate priming of the cells using IL-2 and on a direct physical contact with the fungal pathogen and resulted in the damage of *A. fumigatus*. Using blocking agents and depletion of NK-cell granules, we found fungal killing to be independent of known cytotoxic NK-cell proteins (perforin, granzymes, granulysine), whereas it was clearly dependent on secreted/soluble factor(s). A moderate influence of IFN-γ resulting in fungal damage could be demonstrated using depletion of this cytokine from culture supernatants of activated NK-cells. A whole blood model has been established to characterise the activation of different cell types in a situation close to *in vivo*. By differential FACS staining, activation patterns can be assigned to clearly defined cell populations. In addition, a whole blood model allows us to broaden our understanding of immune responses triggered during sepsis by taking into account yet unstudied populations including NK-cells. Initial analyses include characterisation of the whole blood transcriptome in response to different model pathogens (e. g. *C. albicans, S. aureus).*

Genetic Risk Factors for Invasive Aspergillosis

Invasive aspergillosis (IA) is a life threatening systemic fungal infection in severely immunocompromised patients. With constantly increasing incidence, it has recently become the most relevant fungal infection with regard to mortality rates. The annual cost of treating fungal infections in Europe is currently estimated to be hundreds of millions of Euros annually. In-hospital stays for patients with IA was found to be a median of 17.7 days longer than for uninfected patients

and costs were estimated to be 75000 Euros higher per patient. In the United States, invasive aspergillosis occurs in approximately 10000 patients annually. These numbers underline the medical and socio-economic relevance of *Aspergillus* infections. Patients at risk for the development of invasive aspergillosis are rather well defined and include neutropenic patients and patients with concomitant defects in the T-cell response. Several studies indicate that the individual risk for IA is associated with genetic polymorphisms. Single nucleotide polymorphisms (SNPs) in genes encoding for CXCL10, IFN-γ, IL-10, IL-1β, MBL, plasminogen, TLR1, TLR4, TLR6, TLR9 and TNFR2 have been suggested to be associated with an increased risk for the development of IA in selected patient collectives. However, current information is mainly based on screening for single polymorphisms in small patient cohorts. Furthermore, no analysis of gene copy number variations (CNV) as genetic risk factors has been performed. Therefore the genome-wide genetic background of a high individual risk for IA remains undefined. In the first systematic approach to identify genetic markers associated with IA, genome-wide screening for SNPs and gene copy number polymorphisms in a well defined high-risk cohort of patients with IA and controls will be performed. The resulting data will (i) increase our understanding of the genetic background for IA (ii) allow the design of diagnostic tools to estimate the intrinsic risk of individual patients and (iii) open new ways to analyse pathogenesis and immune response. To collect sufficient clinical samples, Fungal Septomics has brought together a network of clinicians and scientist at more than 16 leading institutions in Germany, Netherlands, Belgium, Austria, Ireland, Wales and Sweden. Coordination of this study is performed in close-cooperation with our co-coordinating study partner at the University of Würzburg.

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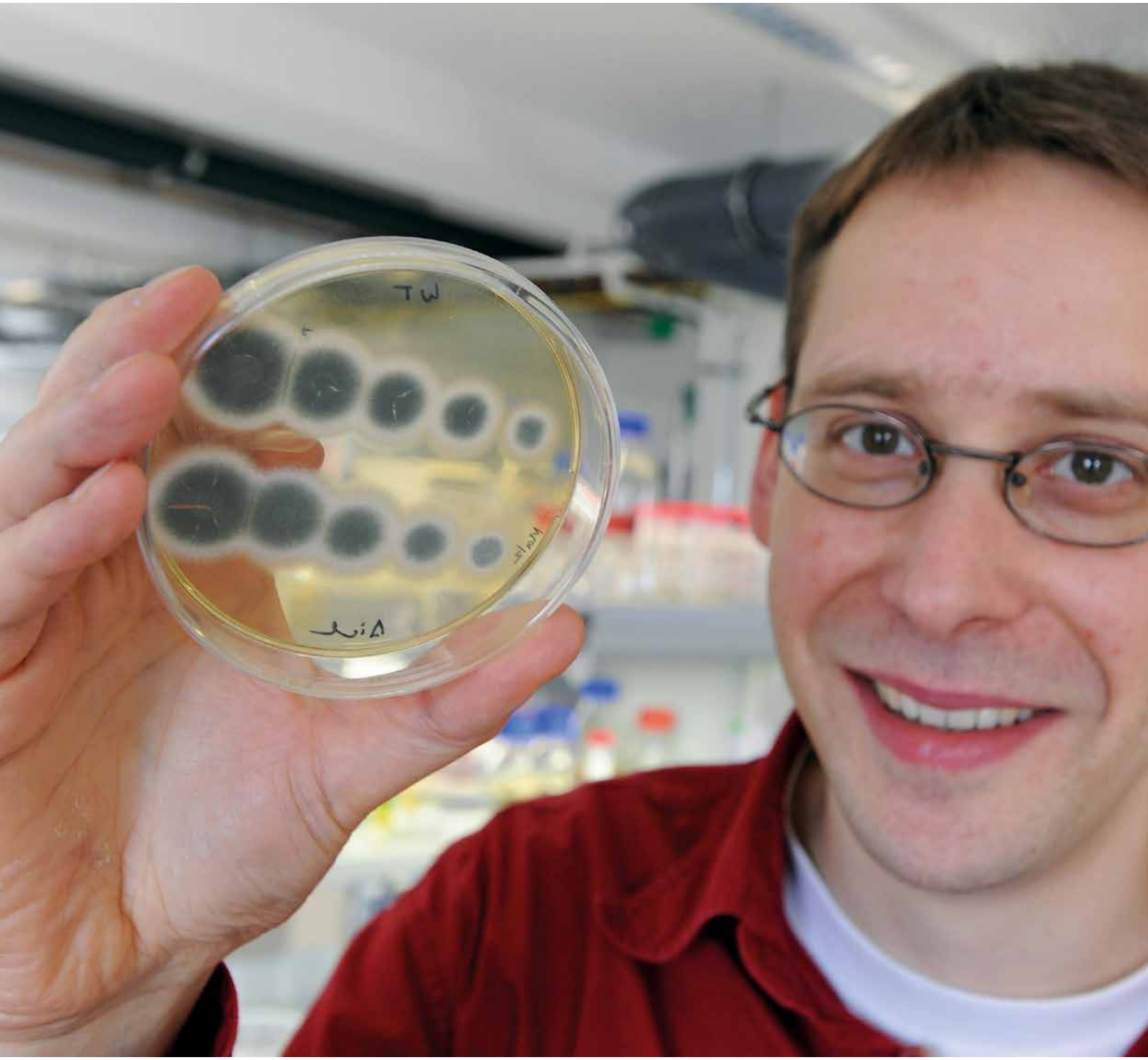

Junior Research Group
Microbial Biochemistry and Physiology

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Microbial Biochemistry and Physiology

Numbers of patients with transient or long lasting immunosuppression are increasing, which is mainly due to improvements in therapy of haematologic malignancies and solid organ transplantation. Imbalances in the immune status cause increased susceptibility for life-threatening invasive fungal infections. Unambiguous diagnosis of fungal infections is difficult and successful therapy is often hampered by restricted efficiency of well-established antimycotics. To advance diagnostic tools and treatment of fungal infections a better knowledge of the metabolic physiology of fungi during the infection process, the identification of general virulence determinants and insights on disease progression in dependence of the host immune status are urgently required.

Work of the Junior Research Group Microbial Biochemistry and Physiology investigates and compares primary metabolism of various pathogenic fungi to draw a more complete picture of the metabolic capacity of fungi. Specific disruptions of essential metabolic pathways can provide new targets for

INTRODUCTION | EINLEITUNG

Head:
Dr. habil. Matthias Brock

Fortschritte in der Medizin zur Behandlung hämatologischer Erkrankungen und in der Organtransplantation führen zu einer steigenden Anzahl von Patienten mit transienter oder lang anhaltender Immunsuppression. Dies führt zu einer erhöhten Suszeptibilität für lebensbedrohliche invasive Pilzinfektionen. Eine eindeutige Diagnose solcher Infektionen ist meist schwierig und die gängigen Therapeutika besitzen häufig nur ein eingeschränktes Wirkspektrum. Um Diagnose und Behandlung von Pilzinfektionen zu verbessern, ist ein Verständnis der metabolischen Physiologie von Pilzen in der Infektionsphase, das Identifizieren allgemeiner Virulenzdeterminanten und ein Verständnis der Progression des Infektionsverlaufs in Abhängigkeit vom Immunstatus von Patienten eine wichtige Voraussetzung.

In der unabhängigen Nachwuchsgruppe Mikrobielle Biochemie und Physiologie beschäftigen wir uns daher mit dem Primärmetabolismus verschiedener Pilzspezies, um ein Bild über die metabolischen Leistungen dieser Organismen zu erhalten. Durch die gezielte Unterbrechung essentieller metabolischer Prozesse könnten neue Antimy-

antimycotics that can act on a wide variety of different pathogenic fungi. Besides investigation of primary metabolism, we also study the production of secondary metabolites. Especially filamentous fungi are well known for the production of useful metabolites that are applied in medicine to treat various diseases. Among them are metabolites such as Penicillin or Lovastatin. However, fungi are able to produce a wide range of metabolites with frequently yet unknown biologic activities and toxic metabolites could contribute negatively to disease progression. Besides these metabolic investigations, we also developed an *in vivo* detection system that allows to monitor disease progression in spatial and temporal resolution by repeated imaging of individual living animals. This tool enables us to study the effect of the route of infection accompanied by the underlying host immune status on disease progression. Additionally, this system allows to study drug efficiency under *in vivo* conditions using small animal numbers. These investigations might help to suggest new treatment strategies to combat life-threatening fungal infections.

kotika gefunden werden, die ein breiteres therapeutisches Spektrum gegenüber einer Großzahl verschiedener Pilzspezies abdecken. Daneben interessieren wir uns für die Produktion von Sekundärmetaboliten filamentöser Pilze. Besonders filamentöse Pilze sind für die Produktion wichtiger Metabolite bekannt, die z.B. in der Medizin zur Behandlung von Krankheiten eingesetzt werden. Hierzu zählen unter anderem die Metabolite Penicillin oder Lovastatin. Daneben kann jedoch auch eine Großzahl bisher teilweise unbekannter Metabolite gebildet werden, die durch toxische Wirkungen den Infektionsprozess negativ beeinflussen können. Neben diesen metabolischen Untersuchungen wurde weiterhin ein *in vivo* Detektionssystem entwickelt, das es ermöglicht, an individuellen lebenden Tieren die Progression von Pilzinfektionen in zeitlicher und räumlicher Auflösung zu studieren. Hierdurch kann der Einfluss der Infektionsroute und des Immunsystems auf den Infektionsprozess in bildgebenden Verfahren verfolgt werden. Dieses System ist daher ebenfalls geeignet, um Wirksamkeitsstudien von Antimykotika in kleinen Tiergruppen durchzuführen und neue Therapiestrategien zu definieren.

1 Evolution of metabolic pathways by gene duplication and specification

Fungi possess an extremely versatile metabolic capacity. Most opportunistic human fungal pathogens such as Aspergilli are ubiquitously present in the environment and feed as saprophytes from dead organic matter. For metabolism of propionyl-CoA that can derive from degradation of propionate, odd-chain fatty acids or certain amino acids filamentous fungi utilise the so-called methylcitrate cycle with its key enzymes methylcitrate synthase, methylcitrate dehydratase and methylisocitrate lyase. By investigating the virulence of a methylcitrate synthase mutant we have previously shown that the ability to efficiently metabolise propionyl-CoA essentially contributes to virulence. Interestingly, the enzymatic reactions present in the methylcitrate cycle are closely related to those of the glyoxylate cycle (Figure 1), which is required for the *de novo* generation of oxaloacetate during growth on gluconeogenic nutrient sources. To elucidate the phylogenetic relationship of key enzymes from both pathways, we selected the respective key enzymes isocitrate lyase and methylisocitrate lyase. Since glyoxylate and methylcitrate cycle are also present in some bacteria, we were interested, whether the fungal enzymes were acquired from bacteria or evolved independently.

In collaboration with the Research Group Systems Biology and Bioinformatics we performed detailed phylogenetic analyses by selecting enzymes from plants, fungi, bacteria and archaea and comparing their phylogenetic relation (Müller *et al.*, 2011). Results showed that fungal isocitrate and methylisocitrate lyases were closely related and grouped with bacterial isocitrate lyases. In contrast, bacterial methylisocitrate lyases appeared not closely related to either one subgroup. Thus, we speculated that fungi acquired an isocitrate lyase, but not a methylisocitrate lyase from bacteria early in evolution. We further speculated that the fungal methylisocitrate lyase evolved from a gene duplication of the isocitrate lyase that subsequently underwent mutations for adaptation to the substrate methylisocitrate. To confirm this hypothesis, crystal structures of isocitrate lyases were investigated to identify amino acids within the active site that could contribute to substrate specificity. A phenylalanine and a threonine were detected in the active site of isocitrate lyases that were replaced by a leucine and a serine in fungal methylisocitrate lyases. This replacement of amino acids was assumed to form a hydrophobic pocket that provides space for the additional methyl-group of methylisocitrate in the active site of methylisocitrate lyases. To confirm our hypothesis, we performed site directed mutagenesis on the isocitrate lyase from the filamentous fungus *Aspergillus fumigatus*, in which either one or both amino acids were replaced with those found in methylisocitrate lyases. These exchanges strongly diminished activity as isocitrate lyase, but especially the double mutation strongly increased the catalytic efficiency as a methylisocitrate lyase. Thus, we were able to conclude that (i) isocitrate lyases were transferred from a bacterial ancestor to the eukaryotic lineage. (ii) Although methylisocitrate lyases are present in both bacteria and fungi and catalyse the same biochemical reaction, their phylogeny is not related. (iii) In fungi the isocitrate lyase underwent a gene duplication that allowed one duplicate to mutate. (iv) Two key mutations altered the specificity to efficiently convert methylisocitrate. (v) The new enzyme was maintained and became an essential part of the methylcitrate cycle, enhancing the metabolic capacity of fungi.

Future work will deal with the evolution of the other enzymes from the methylcitrate

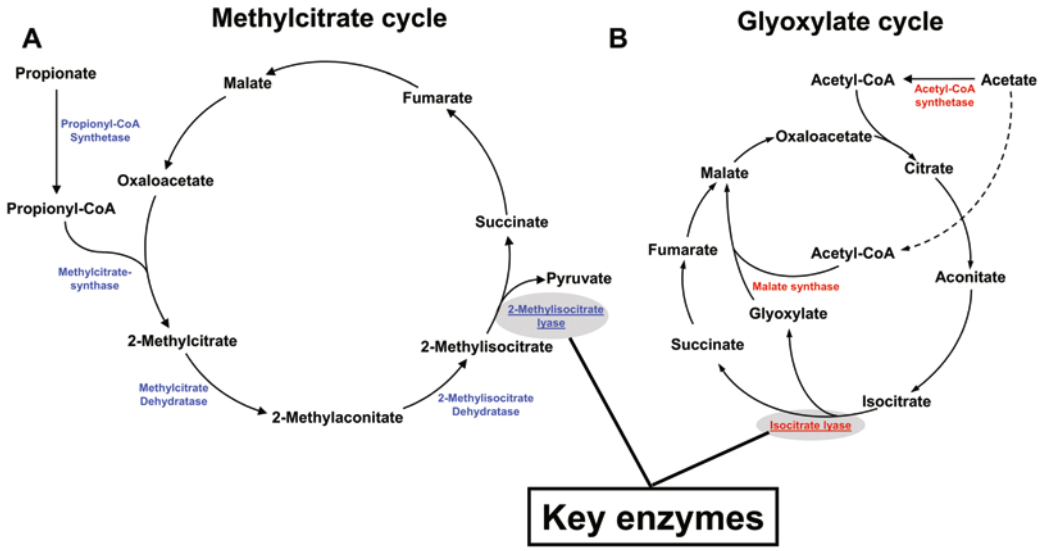

Figure 1
Scheme of the methylcitrate cycle (A) and the gyloxylate cycle (B). The two key enzymes methylisocitrate and isocitrate lyases have been investigated for their phylogenetic relationship.

cycle to identify their phylogenetic origin. In addition, investigations on other metabolic pathways will show, whether gene duplication with subsequent specification is a general mechanism to adapt to a new environment.

2 Morphology directs regulatory mechanisms of metabolism in *Paracoccidioides brasiliensis*

As mentioned above, fungi possess the glyoxylate cycle for the anaplerosis of oxaloacetate during growth on gluconeogenic nutrient sources. Investigations on filamentous ascomycetes have shown that the absence of glucose and the presence of acetyl-CoA generating nutrient sources such as acetate, ethanol or fatty acids are required for induction of gene expression of glyoxylate cycle genes. However, despite its phylogenetic relation to other filamentous ascomycetes, the fungus *Paracoccidioides brasiliensis* employs a different strategy for controlling glyoxylate cycle activity. *P. brasiliensis* is the cause of paracoccidiomycosis with high incidences especially in Latin America. A special feature of this fungus is its ability to undergo a morphologic switch in dependence of the environmental temperature. While growing as a filamentous fungus at temperatures below 30°C, morphology switches to a yeast like form at temperatures above 30°C and exclusively yeast cells are found at 37°C body temperature. Thus, while the infecting structures are generally conidia formed by filamentous structures, the growth form within the infected host is a yeast cell. This switch in morphology may allow a facilitated dissemination from the lung to secondary infected tissues and organs.

Investigations on the transcriptional profile of filamentous cells and the yeast form revealed that filaments possess similar gene expression patterns as known from other fi-

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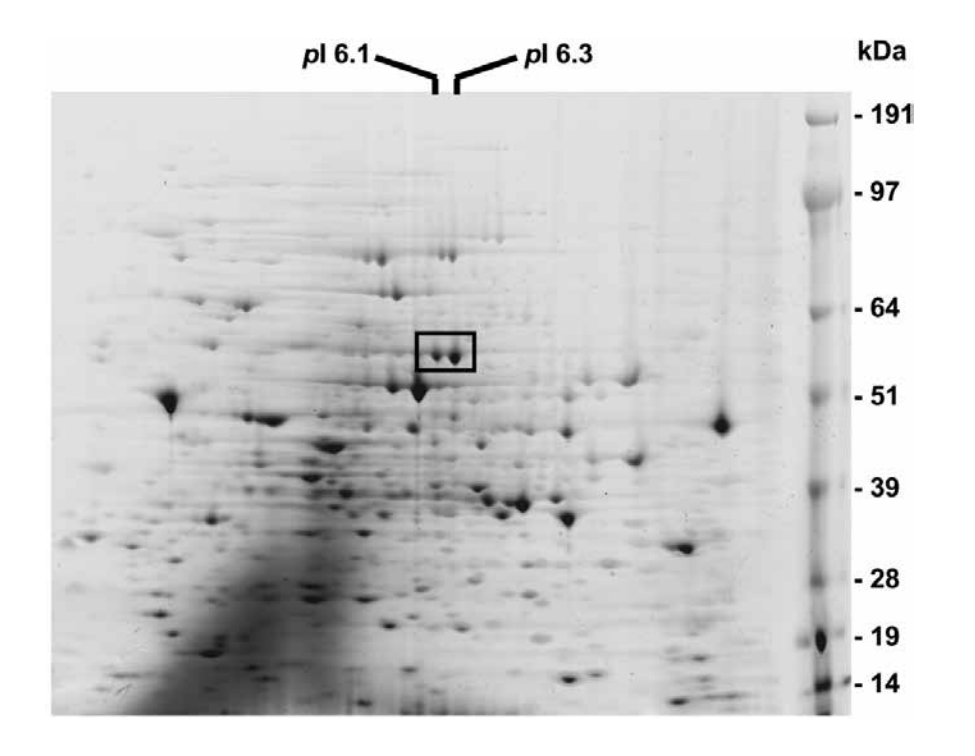

Figure 2
2-D gel analysis of *Aspergillus fumigatus* cell-free extracts after growth on acetate. Two protein spots at different pI values representing the glyoxylate cycle isocitrate lyase were detected (boxed). The shift of the pI value indicates a post-translational modification by phosphorylation. However, in contrast to *Paracoccidioides brasiliensis*, in which phosphorylation of isocitrate lyase provides a mechanism for activity regulation in yeast cells, this modification does not contribute to glyoxylate cycle activity in *A. fumigatus*.

lamentous fungi. However, when cells were grown at elevated temperature, a strong expression of glyoxylate cycle genes was observed even in the presence of the otherwise repressing carbon source glucose.

In collaboration with Prof. M. Pereira (Universidade Federal de Minas Gerais, Brazil), this unexpected phenomenon was investigated in more detail (Cruz *et al.*, 2011). To correlate gene expression with protein production and enzyme activity, yeast cells were grown on different nutrient sources that were assumed to either require a functional glyoxylate cycle such as acetate and ethanol or to be independent from this cycle as assumed for glucose. Investigation of isocitrate lyase gene expression revealed constantly high expression levels independent of the nutrient source. To confirm that isocitrate lyase RNA was indeed translated into protein, polyclonal antibodies were raised against *P. brasiliensis* isocitrate lyase. By using these antibodies in Western blot analyses it was confirmed that isocitrate lyase protein levels were highly abundant regardless the nutrient source. However, when isocitrate lyase activity was determined, activity was high on acetate and ethanol, but only low activity was detected from glucose grown cells. This indicated that a post-translational modification might be involved in regulation of enzymatic activity. To confirm this hypothesis, 2-D gel analyses were performed, in which proteins were blotted from the 2-D gels to membranes for subsequent Western blot analysis with the polyclonal antibodies. Results revealed several spots running at different isoelectric point (pI) values, indicating a modification by phosphorylation. A more detailed analysis showed that especially during growth on glucose spots at more acidic pI values showed higher intensity compared to acetate grown cell extracts. Thus, phosphorylation was assumed to inactivate *P. brasiliensis* isocitrate lyase. Indeed, when extracts of glucose grown cells were

treated with an alkaline phosphatase, isocitrate lyase activity strongly increased and analysis in 2-D gels revealed a spot intensity pattern similar to that from acetate grown cells. In contrast, when extracts from the filamentous fungus *Aspergillus fumigatus* were investigated in 2-D gel analyses, isocitrate lyase was detected only in acetate, but not glucose grown cells. Although a second spot at a more acidic pI value was also observed for *A. fumigatus* isocitrate lyase (Figure 2), treatment with alkaline phosphatase did not change activity. Thus, phosphorylation seems not involved in regulation of isocitrate lyase activity from *A. fumigatus*, but is the major regulatory mechanism for *P. brasiliensis* yeast cells.

One can only speculate on the physiological role of this regulatory mechanism in *P. brasiliensis* yeast cells. However, as indicated above, the yeast form displays the morphological structure found under infectious conditions. Here, fungal cells are faced with rapidly changing nutritional conditions such as the phagolysosome of immune effector cells, the bloodstream or invasive growth within tissues. Due to the fact that isocitrate lyase phosphorylation is reversible, such a regulatory mechanism might allow a more rapid adaptation to changes in the nutritional environment than possible by transcriptional control of gene expression. In conclusion, this example shows that even related fungi have developed different mechanisms to adapt to rapid changes in the nutritional conditions.

3 Growth stage dependent hexose kinase expression in *Aspergillus fumigatus*

Sugars, such as glucose or fructose, are preferred nutrient sources for many microorganisms. Since glucose is also freely available from the bloodstream during infection, glucose can be assumed to play a role for nutrition during pathogenesis. Metabolism of hexose sugars generally starts with an initial phosphorylation of the sugar molecule that leads to substrate activation for subsequent degradation. Thus, hexose kinases are required for sugar utilisation. Genome analyses have shown that several hexose kinases are present in the genome of fungi. While some of these kinases seem to have lost their sugar activating capacity and are, thus, no longer directly involved in sugar metabolism, at least two kinases, called glucokinase and hexokinase, seem to have maintained their catalytic activity. Previous investigations on the model organism *A. nidulans* have shown that deletion of glucokinase does not provoke significant growth defects, whereas disruption of hexokinase reduces the ability of the fungus to utilise fructose. To address the role of these enzymes and their possible redundancy in more detail, we investigated gluco- and hexokinase from the opportunistic human pathogen *Aspergillus fumigatus* (Fleck and Brock, 2010).

In first instance gluco- and hexokinase were produced as recombinant proteins in *Escherichia coli* to perform a detailed analysis on their substrate specificity. Analyses showed that glucokinase possessed a very high affinity to the substrates glucose and mannose, whereas fructose was only activated at minor rates. In contrast, hexokinase showed highest activity with fructose, but was also active with glucose and mannose. Assuming that *A. nidulans* hexose kinases show similar specificities as the *A. fumigatus* enzymes, these analyses were in agreement with the growth defect of the *A. nidulans* hexokinase mutant on fructose. However, to investigate the contribution of these enzymes to sugar utilisation in *A. fumigatus*, both genes were deleted either alone or in combination. When tested for colony formation on solid media containing different nutrient sources the glucokinase mutant behaved similar to the wild type with the exception of some reduced growth on trehalose. In contrast, the hexokinase mutant displayed severe growth defects on all mono- and disaccharides containing fructose. Additionally, conidiation was delayed on nearly all sugars tested. However, the most severe phenotype was observed for

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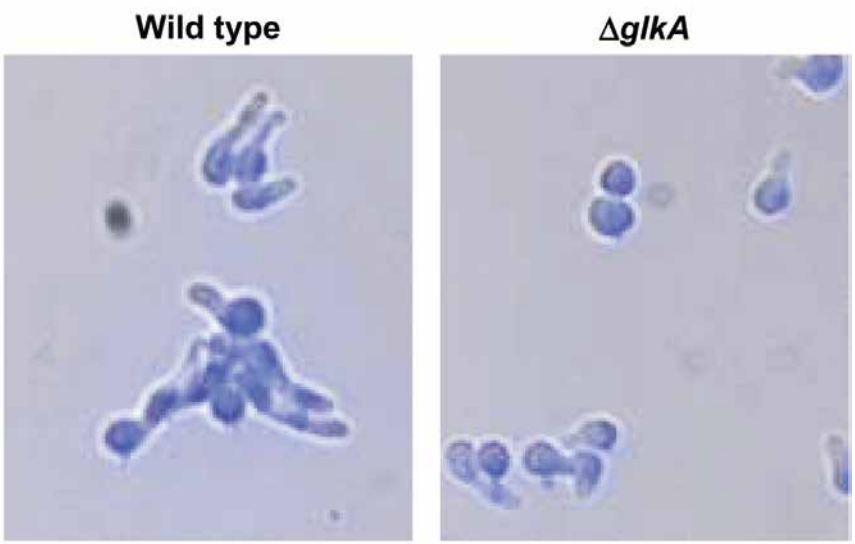

Figure 3
Microscopic analysis of *Aspergillus fumigatus* wild type and glucokinase mutant (Δ*glkA*) after 7 h germination time in the presence of 0.1 mM glucose. While wild-type conidia started to form elongated germ tubes after this incubation time, germination of the glucokinase mutant is significantly delayed.

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Fleck CB, Brock M (2010) *Aspergillus fumigatus* catalytic gluco- and hexokinase: Expression analysis, importance for germination, growth and conidiation. *Eukaryot Cell* 9, 1120-1135.

the double deletion mutant, which showed strongly delayed or no growth on sugars and also displayed growth defects on gluconeogenic nutrient sources. This indicated that (i) glucokinase appears generally dispensable for vegetative growth on sugars; (ii) hexokinase has a dominant function in sugar activation, but activity is supported by glucokinase as (iii) observed from the enhanced phenotype of the double deletion mutant.

However, the obvious redundancy of glucokinase in the presence of hexokinase led to the speculation that this enzyme might possess specific functions under conditions that had not been addressed by the indicated growth experiments. Thus, we went back to the minor growth defect of this mutant observed on trehalose. This disaccharide is one of the major storage sugars in conidia and is cleaved into glucose units early during germination. Thus, a high affinity hexose kinase is required for efficient activation of the minor amounts of glucose released from trehalose. Since glucokinase showed such a high affinity for glucose, we assumed that this enzyme is of special importance for conidia germination. Indeed, analyses of the germination speed (Figure 3) showed a significant delay for the glucokinase mutant that was not observed for the hexokinase mutant. Analyses of the dominating enzyme present in conidia confirmed that glucokinase is the major contributor for glucose phosphorylation. In agreement, RNA levels for glucokinase in conidia were significantly higher than hexokinase levels. In contrast, during germination this ratio inverted showing high levels of hexokinase and low levels of glucokinase.

In conclusion, hexokinase is the dominating sugar phosphorylating enzyme during vegetative growth, which is in agreement with its low specificity for a specific sugar. In contrast, glucokinase evolved as a highly glucose specific enzyme and is required for

efficient and fast germination of conidia. Thus, glucokinase is not a redundant enzyme, but has specified its enzymatic activity for efficient contribution to the metabolic and regulatory network of the germination process. Future analyses will investigate the specific contribution of gluco- and hexokinase for establishment of pulmonal invasive aspergillosis.

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. A multitude of environmental conditions affects the expression of secondary metabolite gene clusters ranging from nutrient composition to oxidative and pH stress or temperature shifts (Figure 4). Besides growing as a saprophyte in soil on dead organic matter, *A. terreus* can cause life-threatening aspergillosis in immunocompromised patients. Here, we have shown in collaboration with Prof. Bernhard Hube and Dr. Ilse Jacobsen (Dept. Molecular Pathogenicity Mechanisms) and Dr. Oumaima Ibrahim-Granet (Unité de recherche Cytokines et Inflammation, Institut Pasteur, Paris, France) that progression of infection proceeds less rapidly than observed for the close relative *Aspergillus fumigatus*, but is accompanied by persistence within immune effector cells. This difference in persistence between *A. fumigatus* and *A. terreus* is mediated by a polyketide synthase producing the metabolite naphthopyrone that is responsible for spore colouration of conidia. *A. fumigatus* produces as final metabolite in the conidia dihydroxynaphtalene melanin that inhibits acidification of phagolysosomes and allows rapid escape of conidia from macrophages. In contrast, although conidia of *A. terreus* are also coloured, the respective polyketide synthase is not present in the genome of *A. terreus*. Thus, phagolysosomes acidify and trap *A. terreus* within macrophages. However, *A. terreus* has a robust survival under such acidic conditions and is, therefore, able to persist for days or weeks within such immune cells (Slesiona *et al.*, 2012a). Besides the ability of *A. terreus* to persist within macrophages, once an acute invasive pulmonal aspergillosis develops, we observed a fatty degeneration of liver hepatocytes (Slesiona *et al.*, 2012b). This implies that toxic metabolites are produced during the infection process.

To identify metabolites that may contribute to virulence, different strategies are possible. One strategy derives from genome mining and direct investigation of a cluster of interest. By this strategy and in collaboration with Prof. Christian Hertweck (Dept. Biomolecular Chemistry) an orphan polyketide-nonribosomal-peptide synthetase (PKS-NRPS) gene cluster was identified in the genome of *A. terreus* (Gressler *et al.*, 2011). To activate the corresponding gene cluster an induced expression of a cluster-specific transcriptional activator was performed, but the cluster remained silent. Thus, reporter strains were generated that allowed to screen for environmental conditions that induce gene expression. A systematic investigation of nutritional conditions revealed that the presence of certain amino acids accompanied with the strict absence of glucose provided conditions for gene cluster expression. Additionally, an alkaline pH had a positive effect on gene expression. These optimised conditions eventually led to the discovery of isoflavipucine and dihydroisoflavipucine as the final end product of the cluster containing the PKS-NRPS hybrid gene. Interestingly, these metabolites belong to a family of fruit rot toxins, implying a supporting function of these secondary metabolites for nutrition in the natural habitat. However, cytotoxicity tests revealed that this metabolite only shows weak biologic activities on a diverse range of mammalian cell lines, indicating that this metabolite is only of minor importance during the infection process. Thus, we are currently investigating the cytotoxic potential of various culture extracts to identify

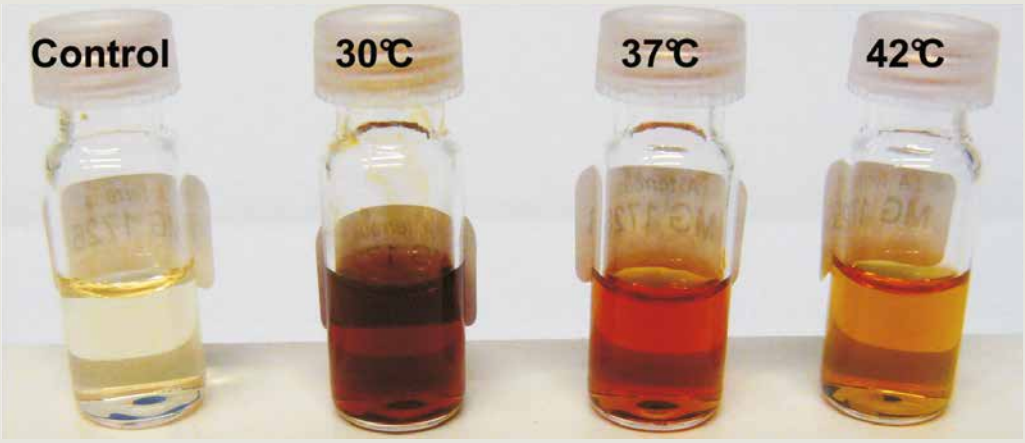

Figure 4
Culture extracts from *Aspergillus terreus* grown for 72 h at different temperatures on potato dextrose broth (PDB). Although the final biomass formed at different temperatures was similar, the amount and composition of extracts varies significantly. The photograph visualises different colour intensities of the extracts that were all solved in a total volume of 1 ml of methanol. The control shows the pure PDB extract without *A. terreus* inoculation.

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metabolites that could be responsible for the damage of liver hepatocytes during the infection process.

5 Bioluminescence imaging for *in vivo* monitoring of the temporal and spatial progression of fungal infections

Conventional virulence studies use large cohorts of animals to draw a picture on disease progression. In such studies animals are generally sacrificed at different time points to obtain snapshots of the current disease status that are finally assembled to create a picture on disease progression. Drawbacks of these conventional analyses can be circumvented by *in vivo* imaging techniques that allow a continuous imaging of the pathogen in individual animals. To apply this technique to fungal infections, a bioluminescence imaging system for monitoring manifestation and progression of disease was developed in collaboration with Dr. Oumaima Ibrahim-Granet and Dr. Gregory Jouvion (Institute Pasteur, Paris, France). For this purpose fungal strains were constructed that express the firefly luciferase under a constitutively active promoter. By that means, the system allows continuous tracking of the location of the pathogen during infection (Brock, 2012).

Aspergilli mainly enter the host *via* the airway, which leads to the development of bronchopulmonary aspergillosis in the immunocompromised host. However, in some cases the infection can disseminate causing high mortality rates due to multiple organ failure. In mice dissemination from the lung to secondary infected organs is difficult to investigate since mice either die from severe inflammation or tissue infarction prior to manifestation of the infection in organs other than the lung. To overcome this problem, conidia can be directly injected into

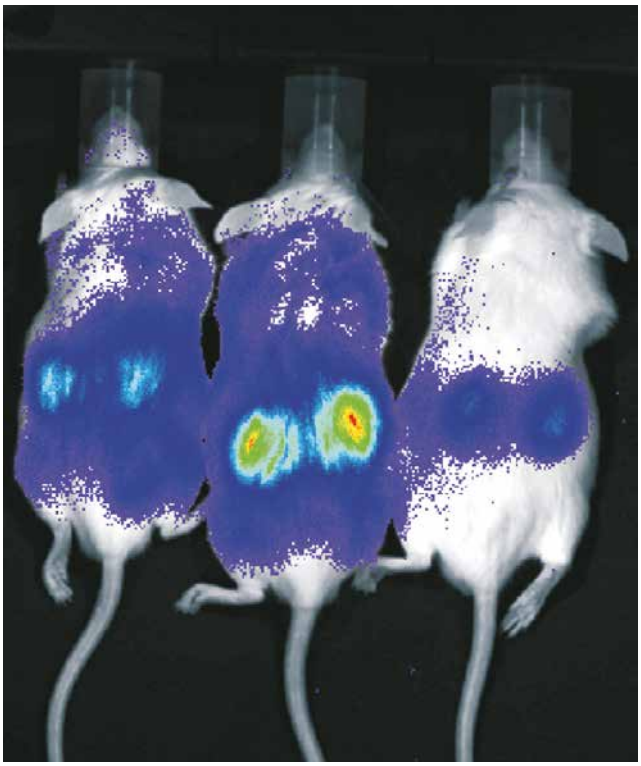

Figure 5
In vivo bioluminescence imaging of corticosteroid treated mice intravenously infected with a bioluminescent *Aspergillus fumigatus* strain. The picture shows a dorsal view on three independent mice 30 h after infection. The colours indicate the amount of photons detected form infected organs. In this view the strong bioluminescence from both kidneys is visible.

the bloodstream to study manifestation of disease in different organs. In our studies, we were interested in the temporal and spatial progression of disseminated aspergillosis in dependence of the host immune status (Jouvion *et al.*, 2012). For this purpose, mice were either immunocompromised by a corticosteroid regimen of left untreated and infected intravenously with a bioluminescent *Aspergillus fumigatus* strain. By following disease progression by bioluminescence imaging (Figure 5), we detected significant differences depending on the host immune status. Corticosteroid treatment led to a rapid progression of disease and animals died within a few days after infection. Bioluminescence imaging implied that the fungus targeted especially kidneys and the liver. Histopathology confirmed bioluminescence imaging results and additionally revealed a marked inflammation within the liver. In contrast, infection of immunocompetent animals showed a much slower progression. Furthermore, bioluminescence signals were exclusively detected from the kidneys as later confirmed by histology. Investigation of the liver of these animals showed no fungal filaments, but some conidia were detected inside the cytoplasm of Kupffer cells. This result indicates that these liver-specific immune cells contribute to controlling liver infections and their function appears attenuated under corticosteroid treatment.

In conclusion, bioluminescence imaging is a valuable tool to follow disease progression under *in vivo* conditions in individual animals. Our investigation clearly indicated the duality between liver and renal tropism of *A. fumigatus* in relation to the immune status of the host. Due to real-time monitoring, differences in temporal and spatial progression of disease are easily visualised and help to draw new conclusions on the infection process. Currently, studies on other fungal pathogens are under way.

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Matthias Brock

Deutsche Forschungsgemeinschaft
Sekundärmetabolismus – *Aspergillus terreus* as a cause of invasive aspergillosis: Impact of secondary metabolites on pathogenesis
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Deutsche Forschungsgemeinschaft
SPP1160 Human-pathogenic Fungi
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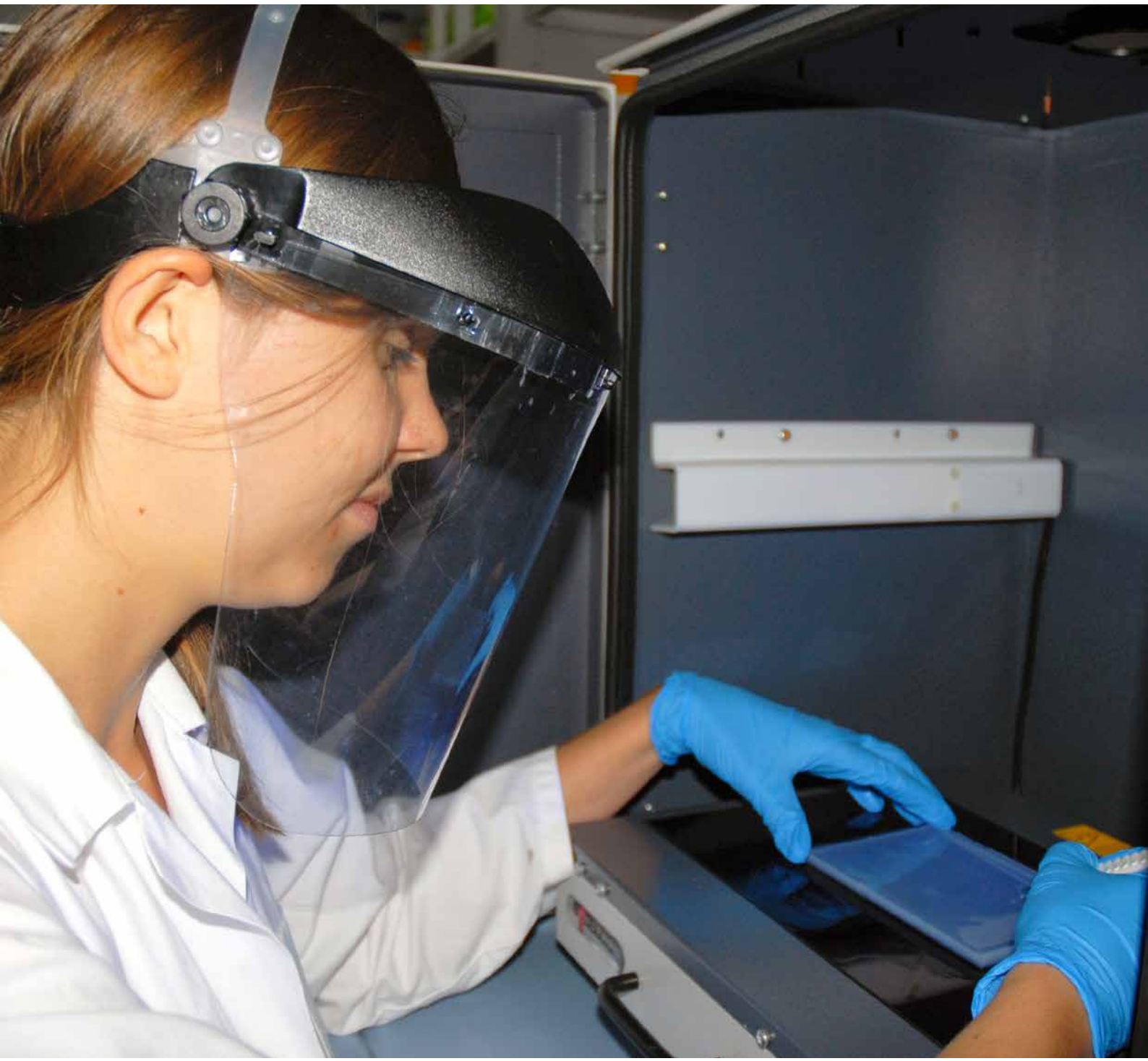

Junior Research Group
Fundamental Molecular Biology of Pathogenic Fungi

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The Junior Research Group Fundamental Molecular Biology of Pathogenic Fungi investigates basic mechanisms of the biology and pathogenicity of human pathogenic fungi, in particular *Candida albicans* and dermatophytes. The opportunistic yeast *C. albicans* is a frequent and harmless commensal of the gastrointestinal and urogenitary tract in healthy people. In the immunocompromised patient however, *C. albicans* can cause mucosal as well as life-threatening, disseminating infections. *C. albicans* rapidly adapts to a variety of environmental conditions and substrates, and also tolerates multiple stresses. These abilities are assumed to be important for the pathogenicity of the fungus, for example during growth and propagation in diverse host niches, or for immune evasion. During these processes, the high flexibility of morphogenetic development also appears to be eminent. Upon distinct host and/or environmental parameters, *C. albicans* can grow as round-oval budding yeasts, filamentous hyphae and large, spherical chlamydospores. In our studies, we identify and functionally address *C. albicans* factors and regulatory pathways, which contribute to these traits. By this approach, we aim to uncover novel pathomechanisms of *C. albicans*, thereby contributing to a better understanding of the complex host-pathogen interaction.

INTRODUCTION | EINLEITUNG

Head:
Dr. Peter Staib

Die Nachwuchsgruppe Molekularbiologische Grundlagen pathogener Pilze erforscht grundlegende Mechanismen der Biologie und Pathogenität krankheitserregender Pilze, insbesondere von *Candida albicans* und Hautpilzen. Die opportunistische Hefe *C. albicans* kommt bei vielen Menschen als harmloser Kommensale im Verdauungs- und Urogenitaltrakt vor, kann jedoch bei abwehrgeschwächten Patienten Infektionen der Schleimhäute sowie lebensbedrohliche, disseminierende Mykosen hervorrufen. *C. albicans* ist in der Lage, sich schnell an variable Umweltbedingungen und Substrate anzupassen und verschiedenste Stressfaktoren zu tolerieren. Diese Merkmale erscheinen für die Pathogenität des Erregers wichtig, beispielsweise für die Nährstoffbereitstellung während der Ausbreitung und Vermehrung in unterschiedlichsten Wirtsnischen oder bei der Vermeidung von Wirtsabwehrmechanismen. Während dieser Vorgänge erscheint auch die Vielseitigkeit in der Morphologie von *C. albicans* als besonders wichtig, die in Abhängigkeit von Wirts- bzw. Umweltsignalen wechseln kann zwischen ovaler, knospender Hefezelle, filamentartiger Hyphe sowie kugelförmiger Chlamydospore. In unseren Studien identifizieren und analysieren wir *C. albicans*-Faktoren und regulatorische Zusammenhänge, die für diese Leistungen wichtig sind. Dadurch sollen neue Pathomechanismen des Erregers aufgedeckt und die komplexen Vorgänge der Erreger-Wirt-Beziehung besser verstanden werden.

Mycoses by dermatophytes are not life-threatening, yet these microorganisms cause the majority of superficial fungal infections in humans and animals, many of which are difficult to cure. The pathogenicity of this specialised group of filamentous fungi is poorly understood, and only a little number of tools are available for their molecular analysis. To better understand the pathogenicity of dermatophytes, key questions need to be addressed, e. g. which determinants of these microbes support the exclusive infection of host structures rich in hard, compact keratin: Hair, skin and nails. Starting from global transcriptional analyses during cutaneous infection and *in vitro* degradation of keratin using the zoophilic species *Arthroderma benhamiae* as a model, we analyse novel, putative pathogenicity mechanisms of dermatophytes. For this approach, we also have adapted straightforward molecular tools in *A. benhamiae*.

Our research should not only contribute to a better understanding of the pathogenicity of *C. albicans* and dermatophytes, but should also help discovering new antifungal targets for the development of future therapeutic strategies.

Hautpilze (Dermatophyten) verursachen zwar keine lebensbedrohlichen Infektionen, jedoch die Mehrzahl der oberflächlichen Mykosen bei Mensch und Tier, die zudem oft schwer therapierbar sind. Die Pathogenität dieser spezialisierten, filamentösen Pilze ist kaum untersucht worden, auch stehen zu ihrer molekularen Analyse nur wenige Werkzeuge bereit. Als besonders interessant erscheint die Frage, welche Virulenzfaktoren dazu beitragen, dass diese Erreger insbesondere Wirtsstrukturen infizieren, die das schwer degradierbare Eiweiß Keratin enthalten: Haut, Haare und Nägel. Ausgehend von globalen Genexpressionsstudien während der kutanen Infektion und des *in vitro* Abbaus von Keratin analysieren wir am Beispiel von *Arthroderma benhamiae* neue, mögliche Pathogenitätsmechanismen von Hautpilzen. Zu diesem Zweck haben wir auch maßgebliche molekulare Werkzeuge etabliert.

Durch unsere Forschungsansätze soll nicht nur die Pathogenität von *C. albicans* und Hautpilzen besser verstanden werden, auch soll letztlich dazu beigetragen werden, neue Zielstrukturen zur Entwicklung neuer Therapieansätze zu entdecken.

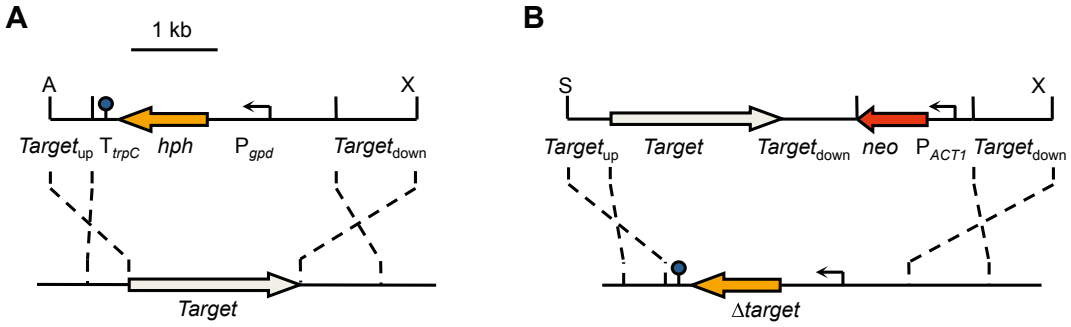

Figure 1
Deletion and reconstitution of specific *A. benhamiae* target genes. **(A)** Structure of the deletion DNA cassette, which contains the hygromycin resistance marker, and genomic structure of a given *A. benhamiae* target gene locus (bottom). The coding region of the target gene is represented by the grey arrow, the upstream and downstream regions by solid lines. The hygromycin resistance gene (*hph*) is shown as an orange arrow, the *gpd* promoter (P_{gpd}) as a bent arrow, the transcription termination sequence T_{trpC} as a filled circle. **(B)** DNA fragment used for reinsertion of the target gene into its original locus in the constructed mutant (Δ*target*, bottom). The neomycin resistance gene (*neo*) is shown as a red arrow, the *A. benhamiae ACT1* promoter (P_{ACT1}) as a bent arrow. Restriction sites given in panels A and B are: A, ApaI; S, SalI; X, XbaI. (modified from Grumbt et al., 2011b).

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1 Functional gene analysis in *A. benhamiae in vitro* and during infection

In order to get novel insights into the pathogenicity of dermatophytes we have focussed our research on the zoophilic species *Arthroderma benhamiae*. The fungus causes highly inflammatory cutaneous infections in humans and animals and offers basic traits which make the microorganism an adequate model for the molecular analysis of dermatophytes (for review see Grumbt *et al.*, 2011a). These advantages include a comparatively fast *in vitro* growth on standard laboratory media, the production of abundant microconidia, the ability to undergo sexual reproduction, and – provided by work of our group – the availability of global gene expression profiles during infection. The recent sequencing approach (a collaboration headed by Axel A. Brakhage, Dept. Molecular and Applied Microbiology) has provided full genome sequence information of the *A. benhamiae* strain which we use in our studies, another important prerequisite for subsequent in-depth genetic research (Burmester *et al.*, 2011). As a major drawback, however, dermatophytes are less amenable to transformation and gene targeting compared with many other fungi. Given the importance of site-directed gene manipulation, we have now established a genetic system for *A. benhamiae* which allows both efficient gene knock-out and reconstitution, by use of linear DNA cassettes containing different dominant selection markers (Figure 1). These efforts have been exemplified by the analysis of a key enzyme of the glyoxylate cycle in *A. benhamiae*, i.e. the putative malate synthase. The factor was shown to be important for the growth of the fungus on lipids, however, no role was observed for cutaneous dermatophytosis in experimental guinea pig infection or during epidermal invasion in a newly established *in vitro* model of reconstituted human epidermis

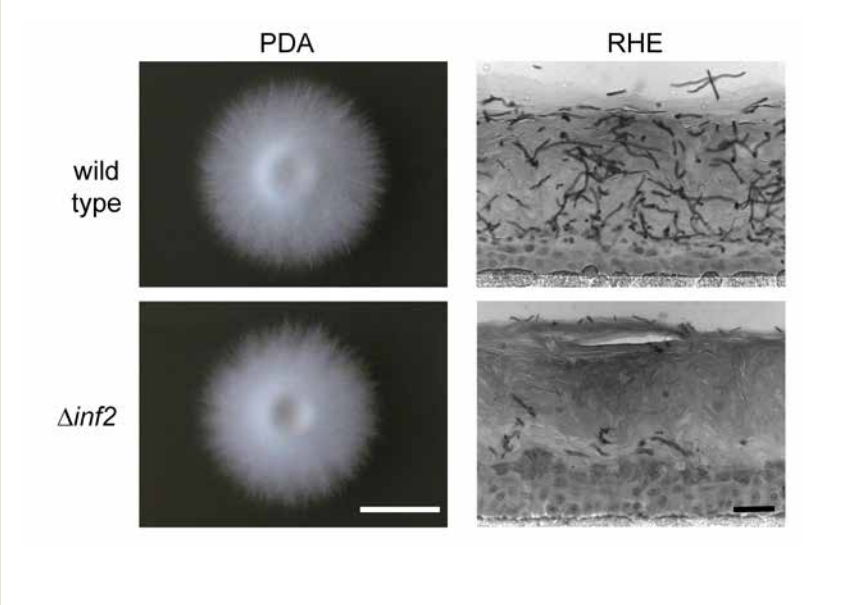

Figure 2
Analysis of *A. benhamiae* genes which are important for infection of reconstituted human epidermis (RHE). Transcriptional profiling of *A. benhamiae* cells during RHE infection identified genes, the deletion of which resulted in impaired fungal growth on the RHE (here exemplified by clone Δ*inf2*), when wild-type cells caused massive tissue invasion and destruction (4 days, 37°C 5% CO_2; scale bar: 50 µm). For control, wild type and mutant were grown for 3 days on potato dextrose agar (PDA) (scale bar: 1 cm). Further details on RHE infection by *A. benhamiae* are given in Grumbt *et al.* 2011b.

(RHE) (Grumbt *et al.*, 2011b). The presented strategy for gene-targeting in *A. benhamiae*, paired with the availability of sophisticated infection models, should advance the future characterisation of putative virulence determinants in dermatophytes.

2 Analysis of infection-specific unknown-function genes and major transcriptional regulators in *A. benhamiae*

We further investigated *A. benhamiae*-host interactions, using the RHE-model to monitor the fungal transcriptome during epidermal invasion. Global microarray analysis and RNA sequencing allowed a comparison with previously obtained data during cutaneous guinea pig infection and *in vitro* keratin degradation (Staib *et al.*, 2010). Selected putatively infection-related *A. benhamiae* genes identified by this approach were next studied in more detail, i.e. by the construction of specific knock-out mutants and subsequent phenotypic analyses. Among others, the project addresses infection-specific unknown-function genes, putative heat-shock factors, transporters, etc., and in addition, a number of selected transcriptional regulators. Herewith included are transcription factors like *stuA*, *steA* and *medA*, putative homologues of which have previously been associated with the morphology, sexual reproduction and/or the pathogenicity of other related filamentous fungi, e.g. *Aspergillus spp.* Preliminary results of mutant analysis already revealed that some of these regulators appear to play key roles during the degradation of keratin substrates or epidermal invasion (Figure 2). Additional phenotypic analyses of mutants and reconstituted strains are currently performed. Since *A. benhamiae* is teleomorphic, these experiments also address mating behaviour and cleistothecia development, a phenotype which we have studied in the

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Symoens F, Jousson O, Planard C, Fratti M, Staib P, Mignon B, Monod M (2011) Molecular analysis and mating behaviour of the *Trichophyton mentagrophytes* species complex. *Int J Med Microbiol* 301, 260-266.

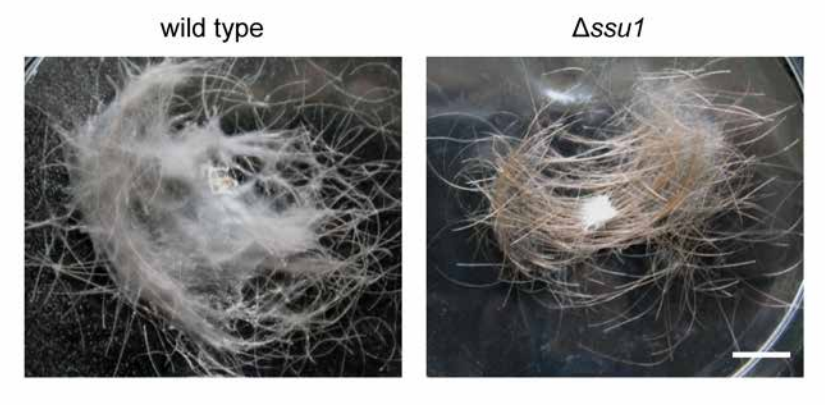

Figure 3
The *A. benhamiae SSU1* gene is important for the growth of the fungus on human hair. Fungal growth/production of woolen mycelia is strongly impaired in case of the Δ*ssu1* mutant after incubation for two months at 25°C (scale bar: 1 cm).

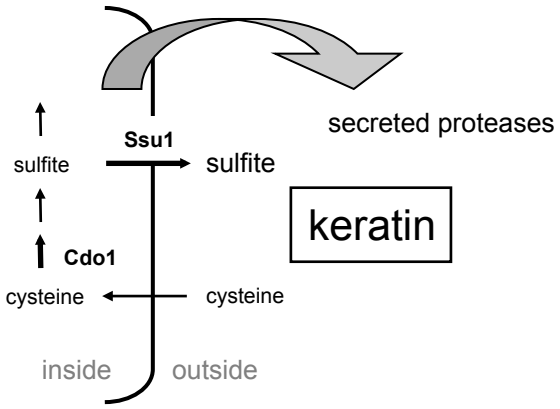

Figure 4
Proposed, simplified model for keratin degradation by dermatophytes. Cysteine from keratin substrates is degraded in the fungal cells via the key enzyme cysteine dioxygenase Cdo1, leading to the formation of sulfite, which in turn is excreted by help of the sulfite efflux pump Ssu1. Sulfite supports the progression of extracellular keratin degradation by secreted proteases.

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Trichophyton mentagrophytes species complex (includes *A. benhamiae*) (Symoens *et al.*, 2011). By this approach, putative biological and/or pathogenicity-related functions of the analysed genes will be defined in more detail, which in the case of transcription factors, include the elucidation of target genes and regulatory pathways.

3 The role of sulfite secretion by *A. benhamiae* for keratin degradation

The ability of dermatophytes to degrade and utilise keratin as a sole carbon and nitrogen source has long been proposed as a key virulence attribute of these fungi, supporting the exclusive infection of keratinized host structures. Already in the early 1970s, a hypothesis has been postulated by which keratin decomposition via dermatophyte secreted proteases is facilitated by the concomitant secretion of the reducing agent sulfite, which is thought to cleave the keratin stabilizing cysteine disulfide bonds (Kunert, 1972). Addressing this issue, we have now revealed that sulfite production and keratin degradation by *A. benhamiae* rely on two eminent factors, cysteine dioxygenase Cdo1 and the putative sulfite efflux pump Ssu1. Mutants in either gene were strongly impaired in growth on hair keratin (Figure 3), and in addition, they were growth sensitive in the presence of elevated concentrations of cysteine. Since keratin is rich in cysteine, the proposed circuit of cysteine degradation to sulfite and subsequent sulfite secretion therefore supports both the tolerance to cysteine and sulfite as well as the progression of keratin degradation (Figure 4). Our findings suggest a major function of the discovered pathway in the pathogenicity of dermatophytes.

4 Regulation of sulfite and cysteine tolerance in *C. albicans*

The results on sulfite production by dermatophytes instigated us to look for similar processes also in the pathogenic yeast *C. albicans*. Moreover, the amino acid cysteine has long been known to be growth inhibiting at higher levels for bacteria, fungi and humans, yet cysteine tolerance has hardly been addressed in microbes. Interestingly, we found that *C. albicans* produces sulfite in the presence of increasing environmental cysteine concentrations, but not the baker's yeast *Saccharomyces cerevisiae*. In order to unravel the basis of sulfite formation and secretion in *C. albicans* under these conditions, we focused on two major issues. Similar to dermatophytes, we first showed by mutant construction and analysis that oxidative cysteine degradation and sulfite formation is relying on the enzyme cysteine dioxygenase Cdg1. Second, we discovered that cysteine induced not only the expression of the *CDG1* gene in *C. albicans*, but also the putative sulfite efflux pump encoding gene *SSU1*. Notably, deletion of the latter resulted in enhanced sensitivity of the *C. albicans* cells to both cysteine and sulfite. Investigating the regulatory circuit of sulfite resistance in more detail, we identified a zinc cluster transcription factor, the absence of which resulted in enhanced sulfite sensitivity. Since sulfite is known to be toxic and a potent reducing agent, its formation from cysteine by *C. albicans* suggests putative roles during host adaptation and pathogenicity.

5 Analysis of morphogenetic development in *C. albicans* and *C. dubliniensis*

C. albicans and *Candida dubliniensis* represent closely related pathogenic yeast species which share multiple phenotypes, including the ability to form true hyphae and chlamydospores. The latter structures are large spherical thick-walled cells which are specifically produced only by these two *Candida* species, i.e. during growth on certain nutrient poor media such as rice extract or corn meal agar. Although this characteristic phenotype has long been used in routine diagnosis for species identification, the biological function of chlamydospores is still unknown, and the morphogenetic regulation of their development has hardly been studied. Based on previous own observations, interestingly only *C. dubliniensis* forms pseudoyphae with abundant chlamydospores on Staib agar (syn. *Guizotia abyssinica* creatinine agar), on which *C. albicans* grows as a budding yeast (Figure 5). In order to provide new insights into chlamydospore formation, we compared the global transcriptional profile of *C. albicans* and *C. dubliniensis* during growth in Staib medium by DNA microarray analysis and RNA sequencing. Since this strategy uncovers genes which are likely important for both filamentation and chlamydospore formation, the putative set of chlamydospore-specific genes was narrowed down by the additional comparative analysis of a *C. albicans nrg1* mutant. This mutant is known for its constitutive filamentous growth due to the lack of the global repressor Nrg1. Interestingly however, previous own work demonstrated that the *C. albicans nrg1* mutant also specifically produces chlamydospores on Staib agar, similar to *C. dubliniensis* (Staib and Morschhäuser, 2005). At present, individual candidate genes are functionally characterised in *C. albicans* and *C. dubliniensis*, for their putative role in chlamydospore development but also with respect to other

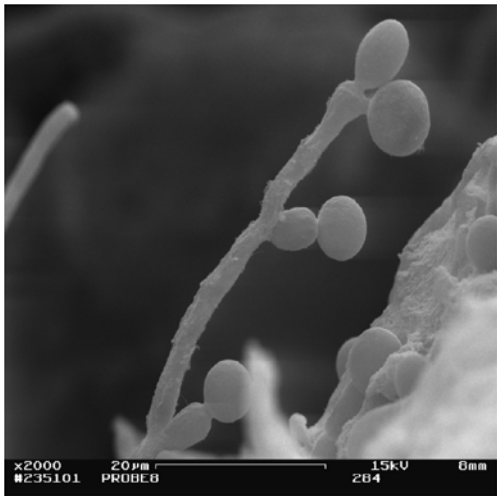

Figure 5
Scanning electron micrograph of *C. dubliniensis* cells grown on Staib agar (syn. *Guizotia abyssinica* creatinine agar) for three days at 30°C. The picture shows mycelia with suspensor cells and chlamydospores.

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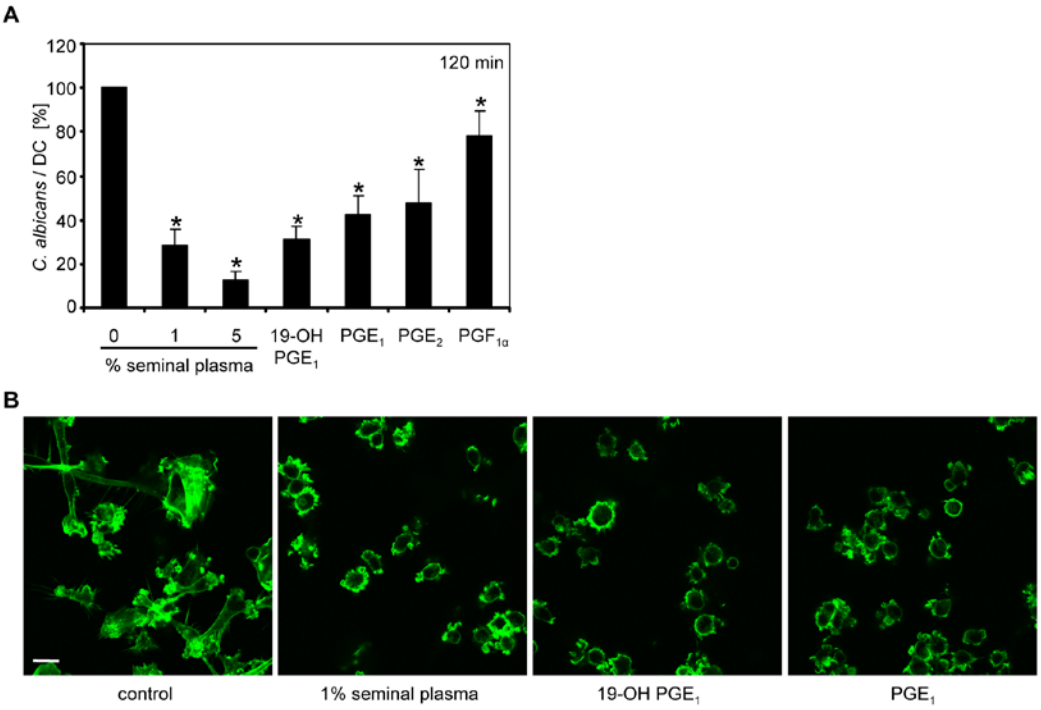

Figure 6

Seminal plasma and specific seminal plasma prostaglandins inhibit the association of *C. albicans* cells with DCs. **(A)** *C. albicans* cells were incubated with DCs for 120 min at 37°C in the presence of 1 or 5% seminal plasma, or 8 µg/ml 19-OH-PGE_1, PGE_1, PGE_2 and $PGF_{1\alpha}$, respectively. After washing and fixation, the number of associated *C. albicans* cells was monitored by microscopy. The percentage of the number of fungal cells binding to DCs in the absence of seminal plasma was set to 100%. The results are the means ± SD from three independent experiments. **(B)** Confocal laser scanning microscopy of DCs in the absence (control) or presence of 1% seminal plasma or 6 µg/ml 19-OH-PGE_1 or PGE_1 for 1 hour. After fixation, the cytoskeleton was stained with phalloidin-FITC (scale bar: 10 µm). Note the altered DC morphology in the presence of seminal plasma or the indicated prostaglandins (modified from Rennemeier *et al.*, 2011).

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phenotypic characteristics. These include also putatively pathogenicity related traits, e.g. stress tolerance, nutrient acquisition, etc. These studies should further elucidate the fundamental biology of these medically important pathogenic species.

6 Host-pathogen interaction and human reproduction

The lower part of the female reproductive tract represents a microbially highly contaminated host niche, thus allowing an integrative view on two major biological processes, human reproduction and host-pathogen interaction. In collaboration with the department of obstetrics and gynecology at the University of Würzburg (Claudia Staib), the present project was started by studying the interaction of human spermatozoa and monocyte derived dendritic cells (DCs). In this context, a putative role of seminal plasma was addressed, since recent evidence suggested that seminal plasma functions not only as a vehicle for sperm, but also as a modulator of female immune defense mechanisms. We observed a strong association of spermatozoa with DCs, a process which was to some extent mediated by the dendritic cell specific adhesion receptor DC-SIGN. Intriguingly, the capture of spermatozoa by DCs was blocked in the presence of increasing concentrations of seminal plasma, accompanied by altered DC maturation and morphology. Investigating the molecular nature of the inhibitory activity of seminal plasma on sperm-DC-association, we identified specific prostaglandins,

in particular PGE_1, 19-OH-PGE_1 and PGE_2, which are known to be present in seminal plasma at high concentrations. We further discovered that *C. albicans*, a common commensal and frequent pathogen of the genital tract, was also protected from the capture by DCs in the presence of seminal plasma or the specific prostaglandins (Figure 6) (Rennemeier *et al.*, 2011). These findings suggested that the immunomodulatory power of seminal plasma may help spermatozoa to escape the attack of DCs of the female reproductive tract, thereby supporting fertilisation. Likewise however, such protective effects of seminal plasma may also modulate the action of DCs during host-pathogen interaction.

In an independent project, which also addresses host-*Candida* interaction, we study the role of a family of secreted aspartic proteases (Saps) of *C. albicans*. These virulence determinants, which are assumed to support nutrient acquisition, tissue invasion, etc. during infection, have long been proposed as putative therapeutic targets. Nevertheless, detailed molecular functions of the various isoenzymes in the pathogenicity of the fungus are still obscure. Since we can produce the individual Saps in *C. albicans* by use of a tetracycline-inducible system, in different collaborations we help to identify novel protease inhibitors and also characterise the interaction of these proteases with certain host cells (Monod *et al.*, 2010; Büchold *et al.*, 2011). These studies should provide novel insights in host-pathogen interaction, especially in view of possible complex influences of soluble factors derived from either host or pathogen.

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Deutsche Forschungsgemeinschaft
Jena School for Microbial Communication
Project: Analysis of environmental signalling and morphogenetic control in the human pathogenic yeast *Candida albicans*
Peter Staib

Deutsche Forschungsgemeinschaft
Jena School for Microbial Communication
Project: Functional analysis of major transcriptional regulators in pathogenic dermatophytes
Peter Staib

Deutsche Forschungsgemeinschaft
Chlamydosporen – Identifizierung und Analyse von Chlamydosporen-und Pathogenitäts-assoziierten Genen in *Candida albicans*
Peter Staib

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Selected publications

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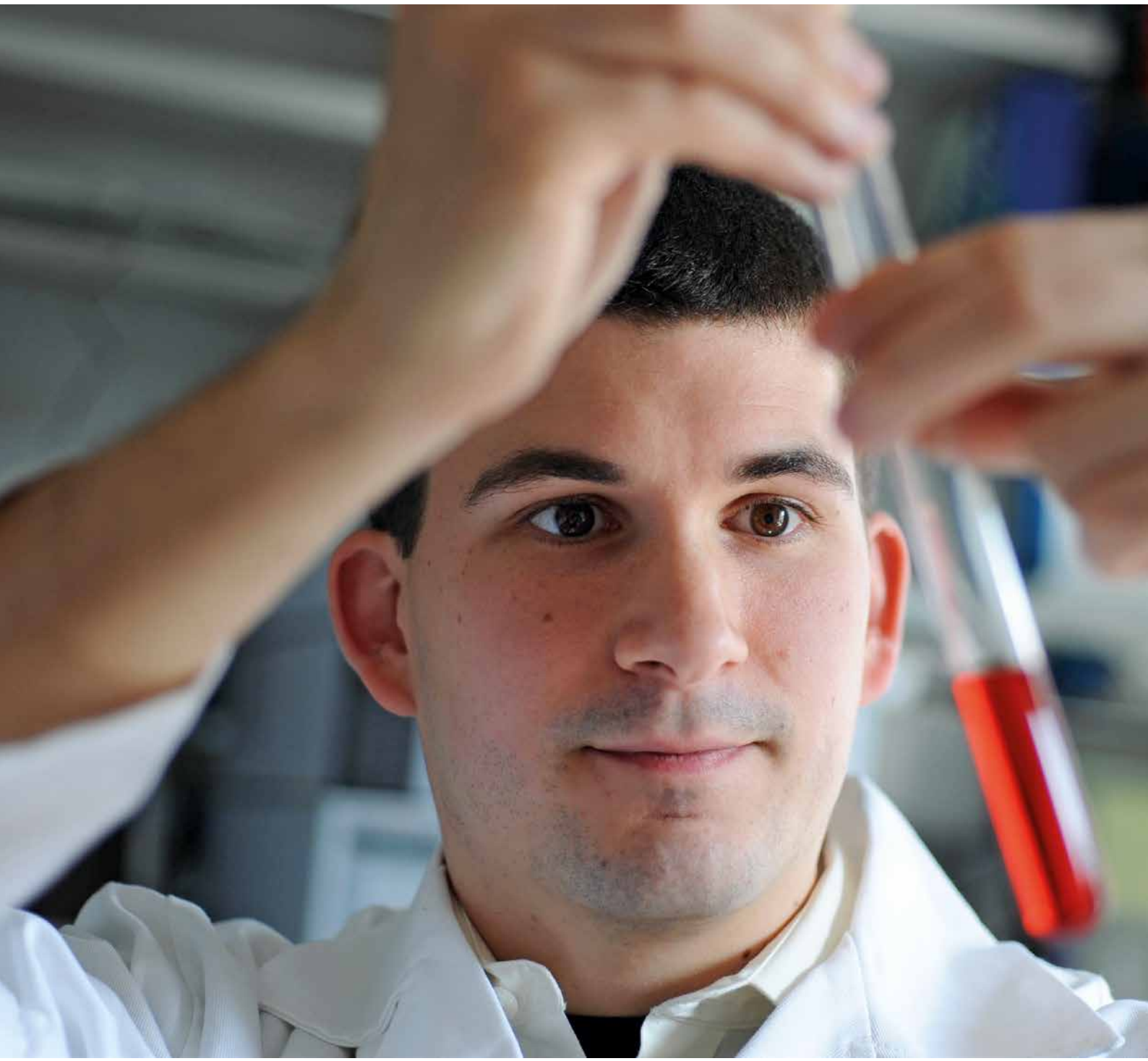

Junior Research Group
Secondary Metabolism of Predatory Bacteria

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Secondary Metabolism of Predatory Bacteria

Natural products continue to be indispensable structural templates for the development of medicinal drugs. They are applied in various therapeutic fields, and many blockbuster drugs, such as the cholesterol-lowering statins or the anticancer agent taxol, actually derive from natural products. The importance of natural products is particularly evident in the treatment of infectious diseases, where they account for the majority of clinically used drugs. Reasons for the broad range of application and the therapeutic usefulness of these compounds certainly are their inherent structural diversity, on the one hand, and the fact that they have been optimised for their affinity to biological targets during evolution, on the other.

In recent years, genomic analyses helped to illuminate the metabolic proficiency of bacteria and fungi, and it turned out that there are many more natural product biosynthetic gene clusters encoded on microbial genomes than previously anticipated. Actually, the total number of identified loci exceeds that of known metabolites by far. Even the genomes of chemically well-studied strains harbour the potential for the production of novel compounds. *Streptomyces griseus*, which is famous for producing the antituberculosis agent streptomycin, has the genetic prerequisite for the assembly of up to 36 different natural products. To date, however, only nine of these metabolites have been described. In case of the bacterium *Saccharopolyspora ery-*

INTRODUCTION | EINLEITUNG

Head:
Dr. Markus Nett

Naturstoffe sind als Leitstrukturen für die Entwicklung neuer Arzneistoffe unverzichtbar. Sie haben vielfältige Anwendungsfelder in der Humantherapie gefunden, und einige der kommerziell erfolgreichsten Wirkstoffe leiten sich von Naturstoffen ab. Beispiele für solche Blockbuster-Arzneimittel sind die Cholesterol-senkenden Statine oder das Antikrebsmittel Taxol. Die Relevanz von Naturstoffen ist besonders evident in der Behandlung von Infektionskrankheiten, wo sie die Mehrzahl der klinisch eingesetzten Wirkstoffe repräsentieren. Gründe für das breite Anwendungsspektrum und den therapeutischen Nutzen dieser Verbindungen sind sicherlich ihre strukturelle Diversität, aber auch die Tatsache, dass sie im Verlauf der Evolution hinsichtlich ihrer Affinität zu biologischen Zielstrukturen optimiert wurden.

In den letzten Jahren hat die zunehmende Verfügbarkeit genomischer Sequenzen neue Einblicke in das metabolische Potential von Bakterien und Pilzen gewährt. Dabei stellte sich heraus, dass die genetisch fixierte Kapazität zur Naturstoff-Biosynthese bei weitem nicht ausgeschöpft wird. Tatsächlich übertrifft die Zahl der auf mikrobiellen Genomen vorhandenen Biosynthesewege deutlich die der bekannten Naturstoffe. Sogar die Genome von chemisch sehr gut untersuchten Stämmen weisen noch Potential für die Produktion neuartiger Verbindungen auf. So besitzt der Produzent des Antituberkulosemittels Streptomycin die genetischen Voraussetzungen für die Assemblierung von bis zu 36 unterschiedlichen Naturstoffen. Bekannt sind bislang aber nur neun dieser Verbindungen. Im Fall des Bakteriums *Saccharopo-*

thraea, which is used for the industrial production of the antibiotic erythromycin, the gap between known and expected chemistry is even bigger (Nett *et al.*, 2009).

Our group is mainly interested in harnessing this idle potential, both for the discovery of new potentially useful drugs and for the study of the biological role of natural products. To retrieve natural products from established sources, we develop and evaluate innovative methods for the structure- and activity-oriented isolation of previously unrecognised metabolites. Furthermore, we investigate microbial groups that are as yet underexplored with regard to their metabolic potential, placing a special emphasis on predatory and phytopathogenic bacteria. We assume that predatory bacteria use natural products as part of their hunting strategy in order to paralyse or kill prey cells. Therefore, they may represent a source of urgently needed antibiotics. In contrast, pests secrete low-molecular weight compounds to promote their virulence. Unveiling the role of natural products in the pathogenicity of select infectious agents will ultimately offer the opportunity to discover new targets for the treatment of plant diseases.

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lyspora erythraea, welches für die industrielle Gewinnung des Antibiotikums Erythromycin eingesetzt wird, ist diese Diskrepanz zwischen bekannter und zu erwartender Chemie sogar noch größer (Nett *et al.*, 2009).

Unsere Gruppe interessiert sich in erster Linie für die Nutzbarmachung des brachliegenden Potentials zur Naturstoff-Biosynthese. Zum einen wollen wir neue, potentielle Wirkstoffe entdecken, zum anderen streben wir ein besseres Verständnis der biologischen Funktion von Naturstoffen an. Für die Findung neuer Naturstoffe aus etablierten Quellen entwickeln und evaluieren wir innovative Methoden, die eine Struktur- und Aktivitäts-orientierte Isolierung bislang übersehender Metabolite ermöglichen. Außerdem untersuchen wir mikrobielle Gruppen, die im Hinblick auf ihr metabolisches Potential bislang nur unzureichend charakterisiert sind, allen voran räuberische und pflanzenpathogene Bakterien. Wir vermuten, dass räuberische Bakterien Naturstoffe einsetzen, um Jagd auf andere Bakterien zu machen. Diese Mikroprädatoren können somit eine Quelle für dringend benötigte Antibiotika darstellen. Demgegenüber fungieren die von Pflanzenschädlingen sezernierten niedermolekularen Verbindungen möglicherweise als Virulenzfaktoren. Die Klärung der Rolle von Naturstoffen für die Pathogenität ausgewählter Schädlinge eröffnet die Möglichkeit, neue Ansatzpunkte für die Behandlung von Pflanzenkrankheiten zu entdecken.

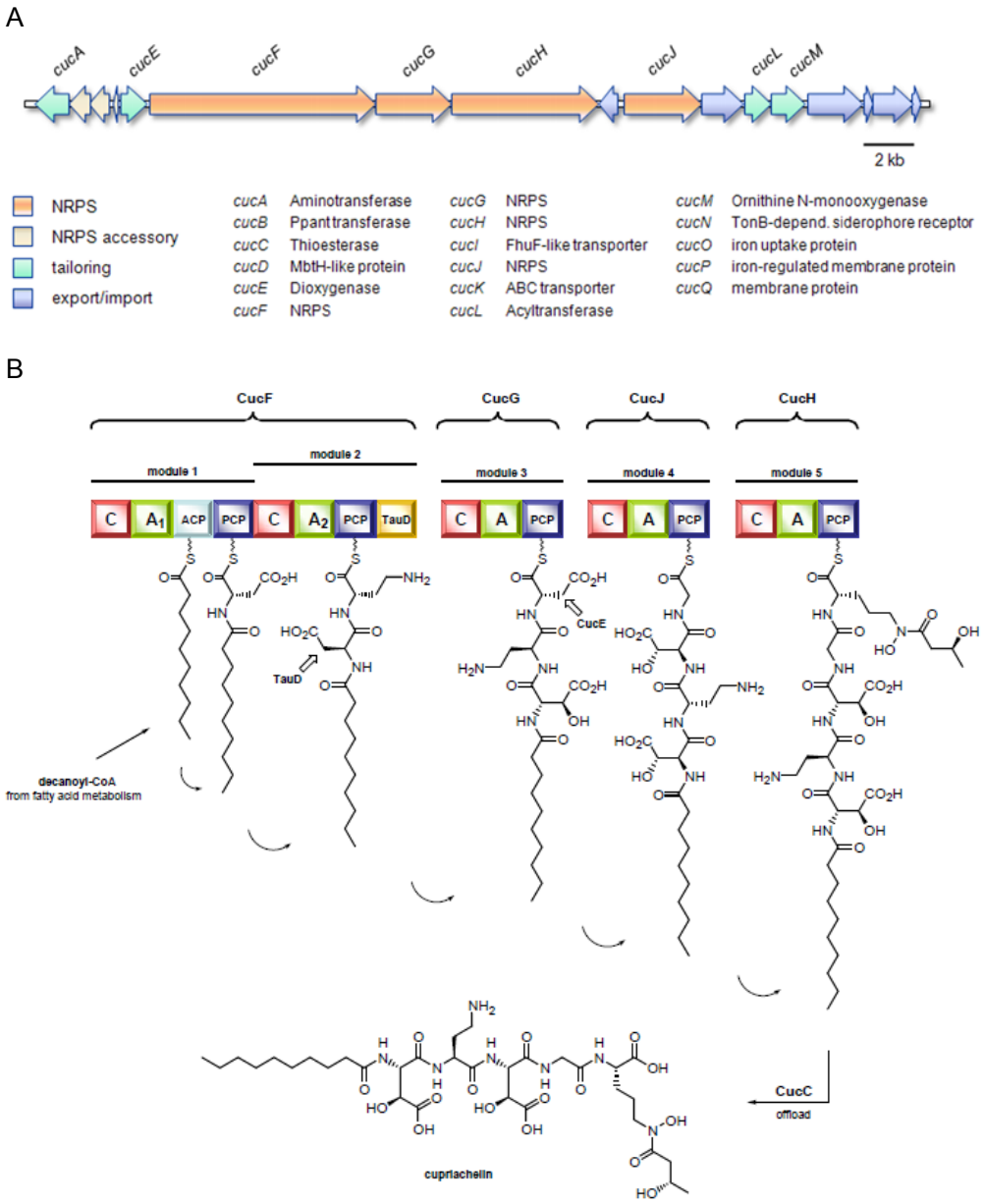

Figure 1
The genetic locus **(A)** and the ensuing assembly line **(B)** for cupriachelin biosynthesis. Domain notation: C, condensation; A, adenylation; ACP, acyl carrier protein; PCP, peptidyl carrier protein; TauD, hydroxylase.

1 A photoreactive siderophore from *Cupriavidus necator*

Cupriavidus necator is a facultative predatory bacterium that was shown to feed on a wide range of microbes. Since literature data suggested the release of a peptidic metal-chelating compound by *C. necator* to promote the killing of prey bacteria, we analysed the genome of the sequenced strain H16 for the presence of genes putatively involved in siderophore biosynthesis. This approach revealed a chromosomal gene cluster that appears to be unique to the investigated strain, lacking homologs even in phylogenetically related bacteria. The architecture of the identified locus suggested a role in siderophore assembly and secretion, featuring genes for the preparation of iron-coordinating functional groups along with lipoprotein receptors and ABC-type transporters. Furthermore, the locus included several open

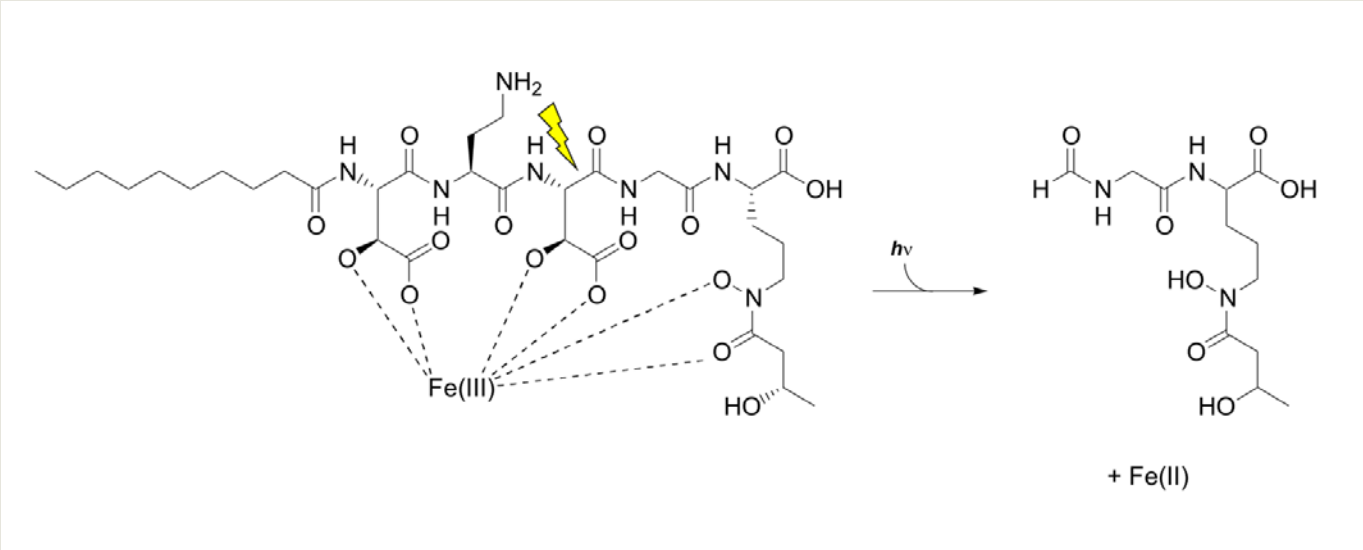

Figure 2
Reaction scheme for the UV photolysis of Fe(III)-cupriachelin.

reading frames, coding for nonribosomal peptide synthetases (NRPS). These enzymes are known to catalyse the linkage of amino acid building blocks in the assembly of peptidic natural products.

Using a genome mining strategy that involved targeted gene inactivation and comparative metabolic profiling of mutant and wildtype strain, we tracked the metabolite that derives from the NRPS gene cluster. The structure of the isolated lipopeptide, which we tentatively named cupriachelin, was fully characterised by spectroscopic methods and its stereochemistry was solved following chemical derivatisation. A model for the biosynthesis of cupriachelin was deduced based upon bioinformatic analyses and the biochemical characterisation of adenylation domain specificity (Figure 1).

Although we did not gather evidence for an involvement in predation, cupriachelin is unique in that it possesses characteristic structural features of siderophores from oceanic bacteria, including a fatty acid moiety and two β-hydroxyaspartate residues. Similar to marine siderophores, cupriachelin is prone to photolysis when coordinated to ferric iron. Exposure to natural sunlight induces an oxidation of its peptidic backbone and a concomitant reduction of the coordinated metal ion (Figure 2). In the oceans, this redox cycling appears to have important ecological implications. By releasing photoreactive siderophores, marine bacteria promote algal assimilation of iron and thus boost algal growth. In return, the bacteria expand their pool of accessible organic carbon. It is obvious that a biological role for cupriachelin's photoreactivity is hardly imaginable in a soil environment. Yet, while most representatives of the species *Cupriavidus necator* are pertained to soil, the cupriachelin-producing strain H16 originates from a creek. The natural occurrence of the bacterium in

a riverine habitat suggests a function for cupriachelin in freshwater ecology analogous to that of marine siderophores, that is the supply of ferrous iron for uptake by planktonic assemblages. The destined release of the siderophore in an aquatic environment would also provide a plausible explanation for its amphiphilic structure. We assume that the inherent surface activity of cupriachelin will slow its diffusion away from the producing bacterium and secure a relatively high concentration around the cell. Comparative genomics lends support towards our hypothesis on the biological role of cupriachelin: the majority of genes of *C. necator* H16 have orthologs in the genome of the recently sequenced *C. necator* strain N-1, but notwithstanding the great degree of synteny between the genomes of the two bacteria, the cupriachelin gene cluster is completely absent in the soil-derived N-1 strain.

2 Natural products from the pest *Ralstonia solanacearum*

The bacterium *Ralstonia solanacearum* is the causative agent of several fatal plant diseases, including Southern wilt of tomato and brown rot of potato. The annual losses due to crop failures are estimated to be around 950 million US-$, making this pest economically relevant. We noticed a strong disproportion between comprehensive genetic research on virulence, on the one hand, and analysis of the secondary metabolome of the bacterium, on the other. This was surprising, as genomic data indicated the potential for the biosynthesis of multiple natural products. The lack of chemical investigations hence seemed to contravene efforts to elucidate pathogenicity mechanisms.

In close collaboration with the research group of Prof. Dirk Hoffmeister (Department of Pharmaceutical Biology, Friedrich Schiller University, Jena), the metabolome of *R. solanacearum* was initially interrogated by inactivating regulatory genes that are known to control the transcription of virulence factors. This approach abolished the production of a family of furanone natural products, whose structures were elucidated (Schneider *et al.*, 2009). In a follow-up study, the genetic locus encoding the biosynthesis of the furanones was identified through a combination of targeted gene inactivation and biochemical reconstitution (Wackler *et al.*, 2011). We then directed our attention onto a chromosomally encoded locus, which is not only conserved among globally dispersed phylotypes of the *R. solanacearum* species complex, but also found, albeit rearranged, on a plasmid of the phytopathogenic bacterium *Burkholderia gladioli*. A bioinformatic analysis revealed a close relationship to the yersiniabactin locus of the plague bacterium *Yersinia pestis*. Although the corresponding genes lack direct sequence similarity, it was evident that the domain architecture of the biosynthesis enzymes is largely conserved in both gene clusters (Figure 3A). We thus speculated that the biosynthetic pathway in *R. solanacearum* codes for a compound that is structurally related to yersiniabactin. But how to track this metabolite? Yersiniabactin is an important virulence factor for many human pathogenic enterobacteria, mediating bacterial iron uptake during infection and limiting the availability of iron to cells of the innate immune system. The natural product is only produced under low iron conditions, since the unregulated uptake of iron can lead to unfavourable intracellular chemistry, e.g. Fenton or Haber-Weiss reactions. Exploiting this knowledge, we subjected *R. solanacearum* to iron deficiency in order to induce the expression of the identified gene cluster. From the comparison with a culture grown in the presence of iron, it was obvious that the bacterium produces at least one additional metabolite at low ferric iron levels. This compound, micacocidin, was subsequently isolated and its structure determined by NMR spectroscopy (Figure 3B). Competition experiments for metal binding as well as cellular uptake assays together with the research group of Hans Peter Saluz (Dept. Cell and Molecular Biology) corroborated a siderophore function for micacocidin (Kreutzer *et al.*, 2011).

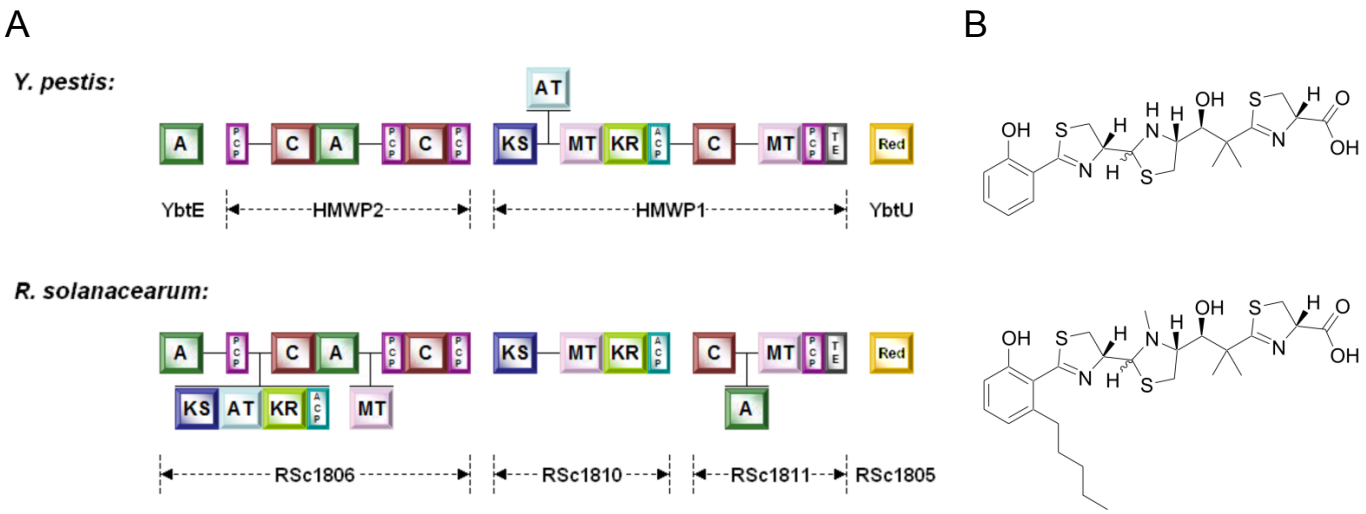

Figure 3
(A) Domain architecture of modular biosynthesis enzymes from two related gene clusters of *Yersinia pestis* and *Ralstonia solanacearum*. **(B)** Chemical structures of the natural products yersiniabactin and micacocidin which derive from these assembly lines.

The confirmation of natural product biosynthesis in *R. solanacearum* provides the basis for future studies that aim at clarifying the role of low molecular weight compounds in plant host-pathogen interactions.

3 Drug discovery in *Streptomyces griseus*

In spite of their metabolic potential, only few new chemical entities are isolated from microorganisms. A common observation, which rationalises this apparent discrepancy, is that many biosynthetic gene clusters remain silent under standard laboratory cultivation conditions. But how can we trigger the expression of silent genes? The answer to this question does not only depend on the potential endogenous natural product producer, but can even be different for two discrete biosynthetic gene clusters in the same organism. This fact still poses a significant challenge to genome mining studies. A very simple, but effective method, which became known as OSMAC (= One Strain – Many Compounds) concept, is the alteration of cultivation conditions. This approach relies on the well known principle that chemical or physical signals, such as phosphate limitation or a pH shift, may exert effects on the transcriptional level of a given microorganism, which in turn may induce the expression of otherwise dormant genes. Together with the research group of Christian Hertweck (Dept. Biomolecular Chemistry), we tested the OSMAC strategy in the bacterium *Streptomyces griseus*, which was consecutively subjected to different fermentation conditions. Depending on the media composition, *S. griseus* produced different sets of natural products, including a peptidic ionophore toxin, various phenoxazinone antibiotics, and an unprecedented quinazoline metabolite (Figure 4; Gomes *et al.*, 2010a; Gomes *et al.*, 2010b; Nett and Hertweck, 2011).

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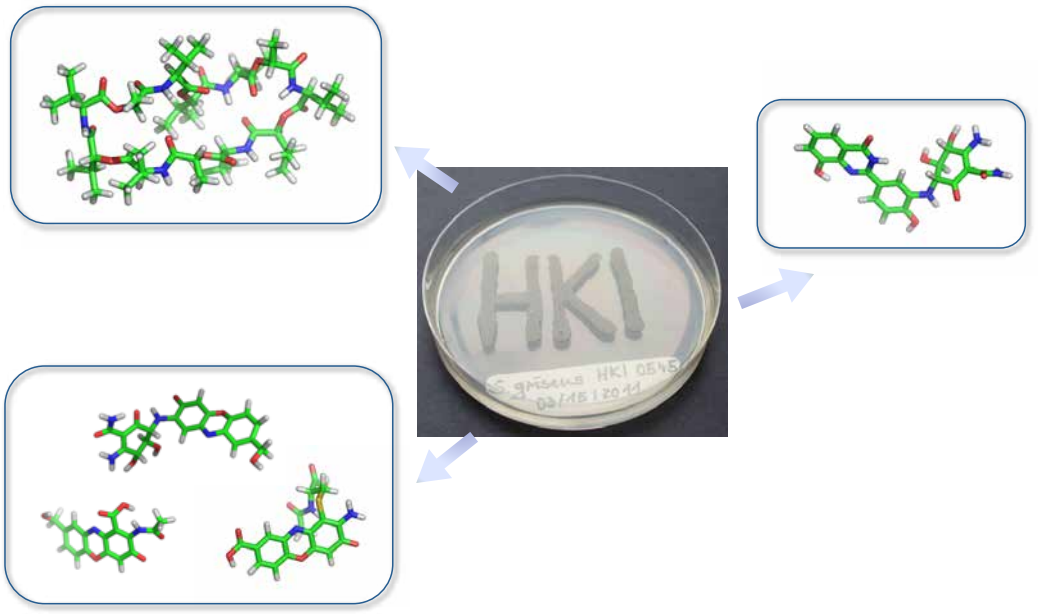

Figure 4
Natural product diversity from *Streptomyces griseus* strain HKI 0545.

In collaboration with the research group of Thomas Henkel (Institute of Photonice Technology – IPHT, Jena) and with Martin Roth (Bio Pilot Plant), we also evaluate the OSMAC approach in customised microfluidic devices, which support the growth of bacteria and fungi in discrete microcultures. To detect the production of bioactive natural products, fluorescent test organisms are added. The growth of the latter can be easily monitored in every compartment of the cultivation chip by automated detection using a microscope with high-speed camera. The lab-on-a-chip (LOC) technology offers the advantages of a high throughput process and will allow the rapid screening of microorganims under varying cultivation conditions.

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Kreutzer MF, **Kage H**, **Gebhardt P**, **Wackler B**, **Saluz HP**, **Hoffmeister D**, **Nett M** (2011) Biosynthesis of a complex yersiniabactin-like natural product via the mic locus in phytopathogen *Ralstonia solanacearum*. *Appl Environ Microbiol* 77, 6117-6124.

Junior Research Group Cellular Immunobiology

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The Junior Research Group Cellular Immunobiology investigates cellular and molecular mechanisms of innate immunity in health and disease. Innate immunity represents an immediately acting defense system against invading microorganisms, but it also participates in inflammation, disposal of cellular debris and in the induction of an effective adaptive immune response. Cells and molecules of innate immunity are able to recognise molecular patterns that are associated with the presence of pathogens. These patterns, such as viral or bacterial nucleic acid, bacterial or fungal surface carbohydrate groups, lipopolysaccharides, are common in certain pathogen groups and are generally not present in the human body.

The number of cases of fungal infections is rising and there is need for effective antifungal drugs. Therefore, we must understand the mechanisms of antifungal immunity in more detail. *Candida albicans* is a common, opportunistic human-pathogenic fungus that can cause superficial and systemic infections in susceptible individuals. We study particularly the activation of human neutrophil granulocytes, major phagocytic cells of innate immunity, upon encounter with *C. albicans*.

INTRODUCTION | EINLEITUNG

Head:
Dr. Mihály Józsi

Die Nachwuchsgruppe Zelluläre Immunbiologie befasst sich mit zellulären und molekularen Komponenten des angeborenen Immunsystems. Das angeborene Immunsystem des Menschen ist verantwortlich für die ersten Abwehrreaktionen gegen eindringende Mikroorganismen. Desweiteren ist es unter anderem an Entzündungsprozessen, der Entsorgung von apoptotischen Zellen und der Einleitung einer effektiven adaptive Immunantwort beteiligt. Zellen und Moleküle der angeborenen Immunität erkennen Gefahrensignale und molekulare Muster, die mit der Gegenwart von Mikroorganismen assoziiert sind. Diese pathogen-assoziierten 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.

Fungale Infektionen treten immer häufiger auf und neue, effektive antifungale Wirkstoffe werden dringend benötigt. Deswegen ist es wichtig, die Mechanismen der antifungalen Immunantwort aufzuklären. *Candida albicans* ist ein opportunistisch humanpathogener Pilz, der bei immunsupprimierten Menschen sowohl oberflächliche als auch schwere systemische Infektionen verursacht. Wir untersuchen insbesondere die

Soluble recognition molecules of innate immunity, such as various complement proteins and pentraxins, recognise both microbes and host structures exposed during physiological or pathological processes, such as apoptotic cells and extracellular matrix components. The research group investigates mechanisms by which the complement system and pentraxins collaborate in the efficient and safe handling of microbes and host material.

Complement is the main humoral component of the innate immune system, with the capacity to destroy microbes. Misdirected complement activation can potentially damage the host; therefore complement activation is strictly regulated. We are interested in malfunctions of complement regulation and investigate the role of anti-complement autoantibodies in human diseases.

zelluläre Antwort von neutrophilen Granulozyten, wichtigen Phagozyten des angeborenen Immunsystems, beim Kontakt mit *C. albicans*.

Lösliche Erkennungsmoleküle, wie z.B. Komplement und Pentraxine, erkennen Mikroorganismen aber auch bestimmte Strukturen des Wirts, z.B. apoptotische Zellen und extrazelluläre Matrix. Unsere Gruppe untersucht die Zusammenarbeit von Komplement und Pentraxinen in dem effektiven und sicheren Umgang mit Mikroorganismen und Wirtsmaterial.

Komplement ist ein wichtiger, löslicher Bestandteil der angeborenen Immunität, welcher in der Lage ist Mikroorganismen zu töten. Eine Fehl-Aktivierung des Sytems kann potenziell zur Schädigung des Wirts führen und wird daher durch eine angemessene Regulation verhindert. Wir interessieren uns für funktionale Defekte in der Komplement-Regulation, und untersuchen die Rolle von anti-Komplement Autoantikörpern in humanen Erkrankungen.

1 Interaction of neutrophil granulocytes with *Candida albicans* and *Candida dubliniensis*

Candida albicans is one of the medically most important human-pathogenic fungi. It is a commensal that can cause superficial and invasive infections in susceptible individuals, e.g. due to genetic predisposition or immunosuppressive treatments. Neutrophil granulocytes are the main effector cells of the innate immune system in antifungal host defense. Neutrophils are quickly attracted to the site of infection and possess a wide repertoire of antimicrobial activities, including phagocytosis, release of extracellular traps, production of reactive oxygen and nitrogen radicals, antimicrobial substances and cytokines.

The pH-regulated antigen 1 (Pra1) has been identified in *C. albicans* as a surface protein, whose expression is upregulated during filamentous growth and which is also secreted by *C. albicans* hyphae. Pra1 has been shown to bind to CD11b/CD18 on phagocytic cells and to mediate cellular adherence. In cooperation with the Department of Microbial Pathogenicity Mechanisms and the Department of Infection Biology, we have investigated the role of Pra1 in the interaction of *C. albicans* with human neutrophil granulocytes. Neutrophils showed reduced adhesion to and phagocytosis of fungal cells lacking Pra1 compared to the wild-type strain, whereas the formation of extracellular traps was not affected. Similarly, we observed reduced respiratory burst and release of lactoferrin and interleukin 8 from neutrophils stimulated with the Pra1-deletion mutant in comparison with the wild type. Interestingly, Pra1-overexpressing *C. albicans* induced stronger neutrophil responses than the wild type only when they were inactivated with thimerosal prior to co-culturing them with the human cells. Live fungal cells overexpressing Pra1 reduced the activation of neutrophils, suggesting that soluble Pra1 released by these cells may act in an inhibitory manner. To analyse the effect of Pra1 released by the fungus, we used recombinant Pra1. Recombinant Pra1 bound to CD11b/CD18 on human neutrophils and inhibited the neutrophil responses triggered by surface-associated Pra1. These results suggest a dual role for Pra1 in the interaction of *C. albicans* and neutrophils. While Pra1 on the fungal surface is recognised by neutrophil granulocytes and triggers cellular activation, *C. albicans* can suppress some of the neutrophil responses by releasing Pra1 and blocking receptors on these immune cells (Figure 1).

C. dubliniensis is a recently recognised close relative of *C. albicans*. Despite the close relatedness of the two fungal species, *C. dubliniensis* is less virulent than *C. albicans*. There is little known about the interaction of *C. dubliniensis* with the human immune system. Therefore, in cooperation with the Junior Research Group Fundamental Molecular Biology of Pathogenic Fungi, we compared the activation of neutrophils during their interactions with *C. albicans* and *C. dubliniensis*. Our data indicate that early neutrophil responses, such as migration, phagocytosis, release of lactoferrin and interleukin 8, and the generation of reactive oxygen species are enhanced upon stimulation with *C. dubliniensis* isolates compared with *C. albicans*. On the other hand, *C. dubliniensis* cells caused less neutrophil damage and extracellular trap formation than *C. albicans*. These data suggest that human neutrophils may be more efficient in the elimination of *C. dubliniensis* than *C. albicans*, thus providing insight into the differential virulence of this two closely related fungal species.

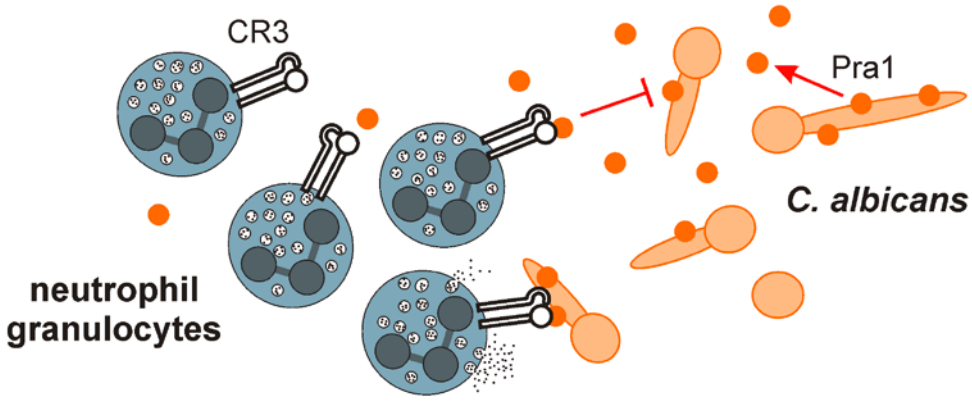

Figure 1
Dual role of the *Candida albicans* Pra1 protein in the interaction with human neutrophil granulocytes. The expression of Pra1 (pH-regulated antigen 1) protein in *C. albicans* is upregulated during filamentous growth and the fungus also releases this protein. Pra1 binds to complement receptor 3 (CR3) on human neutrophils. Pra1 exposed on the surface of *C. albicans* enhances neutrophil adhesion, the generation of reactive oxygen species and the release of antimicrobial substances (e.g., lactoferrin) and interleukin 8. However, released Pra1 can block CR3 on neutrophils and thus interfere with the antifungal activity of these innate immune cells.

2 Anti-complement autoantibodies in kidney diseases

The complement system is a major humoral component of innate immunity which, in addition to its role in eliminating microorganisms, is involved in inflammatory processes, waste disposal, tissue regeneration and regulation of adaptive immune responses. Complement is composed of more than 30 proteins, which are present in body fluids and on cell membranes. Complement activation is initiated by target recognition via three activation cascades, which merge in a common terminal pathway and can lead to the lysis of microbes. On the other hand, complement activation is controlled by several fluid-phase and cell membrane regulators in order to prevent damage to host tissues. Misdirected complement activation and impaired complement regulation are associated with various diseases, including the kidney disorders atypical hemolytic uremic syndrome and dense deposit disease. In this project, we are investigating how autoantibodies against complement proteins disturb the delicate balance between complement activation and inhibition, thus leading to pathological processes.

Anti-factor H autoantibodies are detected in approximately 10 % of patients with atypical hemolytic uremic syndrome. Factor H is a plasma protein that regulates activation of the alternative complement pathway. It is built up from 20 domains, of which the four N-terminal domains mediate the complement inhibitory activity of factor H and the two C-terminal domains mediate binding to host cells. Thus, factor H can also protect host cells from complement-mediated damage. In collaboration with the Department of Infection Biology, we have previously determined the binding sites of autoantibodies within factor H. The analysed autoantibodies all bound to the most C-terminal domain 20

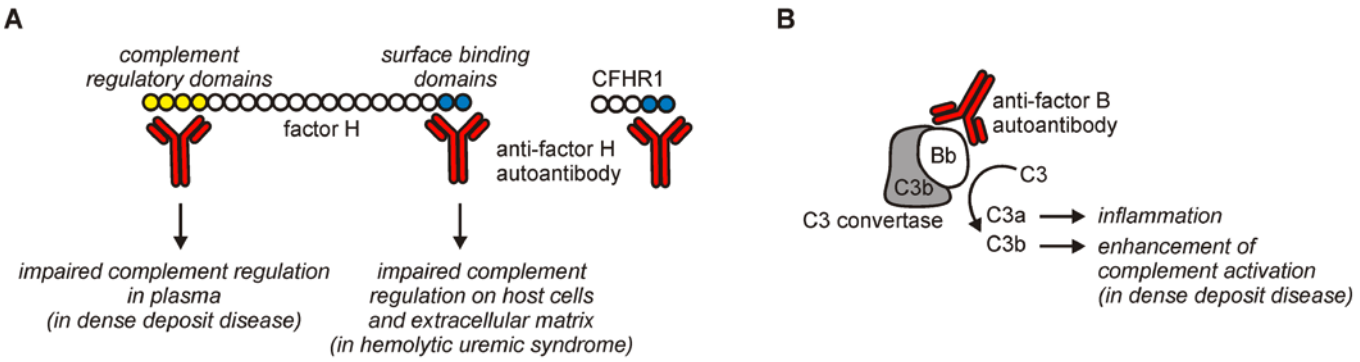

Figure 2

Role of anti-factor H and anti-factor B autoantibodies in the pathogenesis of kidney diseases. **(A)** Autoantibodies to the complement regulator factor H are described in the kidney diseases dense deposit disease and atypical hemolytic uremic syndrome. Factor H is composed of twenty domains. The N-terminal four domains (shown in yellow) are responsible for the complement regulatory activities of factor H and the C-terminal two domains (shown in blue) are responsible for the binding of factor H to host surfaces, thus factor H also protects host cells from complement-mediated damage. Autoantibodies impair the regulatory functions of factor H in plasma leading to dense deposit disease or inhibit the surface binding domains of factor H leading to impaired protection of host cells in hemolytic uremic syndrome. In addition, hemolytic uremic syndrome-associated factor H autoantibodies cross-react with factor H-related protein 1 (CFHR1), because its two C-terminal domains are almost identical with those of factor H. **(B)** We have discovered a new autoantibody in dense deposit disease. This autoantibody binds to complement factor B and its fragment Bb, which is the enzymatically active part of the alternative pathway C3 convertase C3bBb. The convertase-bound autoantibody increases the stability of the convertase, thus it can amplify complement activation by generating increased amounts of C3b fragments.

of factor H and thus interfered with the host cell protective function of this complement inhibitor.

This domain is homologous to the C-terminus of factor H-related protein 1 (CFHR1), with only differences in two amino acids. Therefore, we have analysed whether anti-factor H autoantibodies cross-react with CFHR1. In cooperation with Dr. Pilar Sánchez-Corral's group (Hospital Universitario La Paz Madrid, Spain), we found that 21 of 24 analysed anti-factor H IgG autoantibodies recognised both factor H and CFHR1. For the first time, we described autoantibodies of the IgA isotype, similarly cross-reacting with CFHR1, in three patients. Analyses of IgG fractions from patients showed that native factor H and CFHR1 are indeed present in the immune complexes *in vivo*. In an *in vitro* host cell protection assay, plasma samples of autoantibody-positive patients caused lysis of sheep erythrocytes because they inhibited factor H binding and thus its protective activity on host cells. Addition of recombinant CFHR1 rescued the erythrocytes from lysis by neutralizing the autoantibodies. These data indicate that exogenous CFHR1 during plasma therapy of the patients help in the treatment of this autoimmune form of hemolytic uremic syndrome.

We have also characterised a factor H autoantibody from a patient with dense deposit disease, in cooperation with Dr. Margarita López-Trascasa (Hospital Universitario La Paz Madrid, Spain). Dense deposit disease is associated with uncontrolled complement activation in plasma which leads to kidney damage. This autoantibody bound to the N-terminal complement regulatory domains of factor H. The purified autoantibody inhibited the complement regulatory activity of factor H in a fluid-phase assay. Thus, autoantibodies interfere with different domains of factor H, causing impaired complement reg-

ulation in plasma in the case of dense deposit disease and impaired regulation on host cell surfaces in hemolytic uremic syndrome (Figure 2A).

In addition, we have described and functionally characterised a new autoantibody in dense deposit disease. This autoantibody binds to the complement protein factor B, which is a serine protease. Bb, a proteolytic fragment of factor B, together with complement C3b forms the C3 convertase enzyme of the alternative complement pathway (C3bBb). This enzyme cleaves C3 molecules into C3a and C3b, and the resulting C3b can further propagate the complement cascade. The C3 convertase has a naturally short half-life and it decays spontaneously but also through the action of complement regulators, such as factor H. We found that the anti-factor B autoantibody binds to and stabilises the C3 convertase and leads to prolonged convertase activity. In addition, the autoantibody makes the convertase resistant to extrinsic, e.g. factor H-mediated, decay. In conclusion, this newly described autoantibody enhances complement activation in dense deposit disease (Figure 2B).

This project was supported by a grant from the Deutsche Forschungsgemeinschaft (JO 144/1-1).

3 Crosstalk between pentraxins and the complement system: Interaction of pentraxin 3 (PTX3) with complement regulatory proteins

Pentraxins are soluble recognition molecules of the innate immune system. The prototypic short pentraxin CRP is an acute phase protein produced in the liver. Its levels are strongly elevated in human plasma after trauma or infections, and CRP is a nonspecific marker of inflammation. The long pentraxin PTX3 is produced locally by several cell types, such as macrophages, dendritic cells and endothelial cells, and its elevated plasma levels mirror local inflammatory processes. Pentraxins recognise ligands exposed on microbes and on damaged host cells, such as phosphocholine, phosphoethanolamine, histones and chromatin. CRP and PTX3 activate the complement system, which can lead to deposition of complement proteins on the surfaces where the pentraxins are bound (opsonisation), such as microbes and apoptotic/necrotic cells, and promote clearance through uptake by phagocytes. Excessive complement activation, however, would damage surrounding tissues and may cause lysis of apoptotic/necrotic cells, thus promoting inflammation and autoimmunity. CRP has been shown to bind the complement regulators factor H and C4b-binding protein (C4BP), thus it was speculated that while CRP initiates opsonization, the simultaneous binding of the inhibitors prevents an overactivation of complement. In this project, we investigated the interaction of PTX3 with complement regulatory proteins.

C4BP showed a strong binding to PTX3 (K_d ~5 nM). PTX3-bound C4BP retained its complement regulatory functions. PTX3 bound to apoptotic cells, activated complement and enhanced C3 fragment deposition. However, it also recruited functionally active C4BP that led to inactivation of the C4b protein and attenuation of terminal pathway activation. In addition, we analysed the interaction of PTX3 and complement on extracellular matrix, which represents a host structure exposed to body fluids during tissue injury or damage of the endothelium in blood vessels. We found that PTX3 binds to subendothelial extracellular matrix *in vitro*, and recruits the complement inhibitors factor H and C4BP. While PTX3 triggers complement activation on extracellular matrix by binding C1q, activation of the terminal complement pathway is not increased. Functional assays demonstrated that factor H and C4BP facilitated the inactivation of C3b and C4b, respectively, thus down-regulating complement activation. Interestingly, we found that the complement regulators did not compete with each other for PTX3-binding. Thus, they cooperate with PTX3 in the regulation of lo-

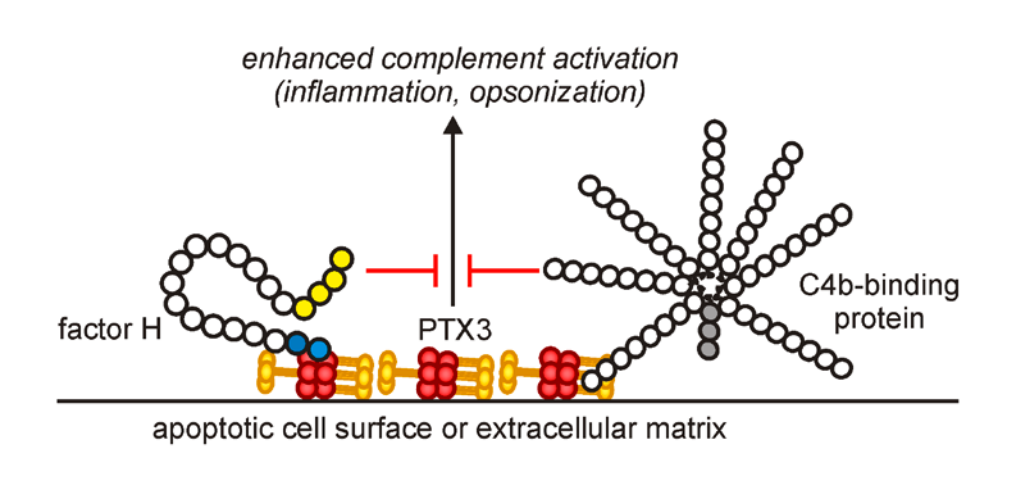

Figure 3
Pentraxin 3 (PTX3) directs the complement inhibitory activities of factor H and C4b-binding protein to biological surfaces. PTX3 recognises certain microbes but also interacts with host structures, such as apoptotic cells and extracellular matrix proteins. When surface-bound, PTX3 can activate complement by interacting with molecules initiating the classical and lectin complement pathways. We demonstrated that PTX3 recruits the complement inhibitory molecules factor H and C4b-binding protein, which in turn down-regulate complement. This balanced interaction of PTX3 with both activatory and inhibitory molecules of complement may ensure optimal and safe opsonization and target clearance but prevent excessive complement activation that would otherwise lead to host tissue damage and inflammation.

cal complement activation and complement-mediated inflammation (Figure 3).

Mutations in factor H and anti-factor H autoantibodies are associated with the kidney disease atypical hemolytic uremic syndrome, which is characterised by damage to the glomerular endothelium. Therefore, we have analysed whether these factor H abnormalities influence the interaction of factor H with PTX3. The tested factor H mutants and some of the autoantibodies caused reduced binding of factor H to PTX3, indicating that such an impaired interaction may contribute to defective local complement regulation on subendothelial extracellular matrix.

We propose that the interaction of PTX3 with complement regulatory proteins is important to promote safe opsonization and clearance of microbes and apoptotic cells, and also contributes to the control of complement activation on host structures such as the extracellular matrix and basement membranes.

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Cross-sectional Unit Bio Pilot Plant

Cross-sectional Unit Bio Pilot Plant

The research of the HKI Bio Pilot Plant is mainly focused on the development, optimisation 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 droplet-based nano-litre scale were developed. This so-called microfluidic technology provides the high-throughput detection of antimicrobial activity in complex biological samples, particularly of 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 optimise 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. Last year, for example, an ultra-HPLC method for the high specific detection of amino acids was developed. Our research strategy is characterised by

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 3.000 l Nettovolumen sowie entsprechend großvolumige Aufarbeitungsmöglichkeiten zur Verfügung. Außerdem wurden Technologien für die Kultivierung von Mikroorganismen im Tropfen basierenden Nanoliter-Maßstab entwickelt, die für die Hochdurchsatz-Entdeckung komplexer antibiotischer Aktivitäten, insbesondere von 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. So wurde z. B. im letzten Jahr eine hochsensitive Ultra-HPLC-Methode für den hochspezifischen Nachweis von Aminosäuren entwickelt.

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.

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.

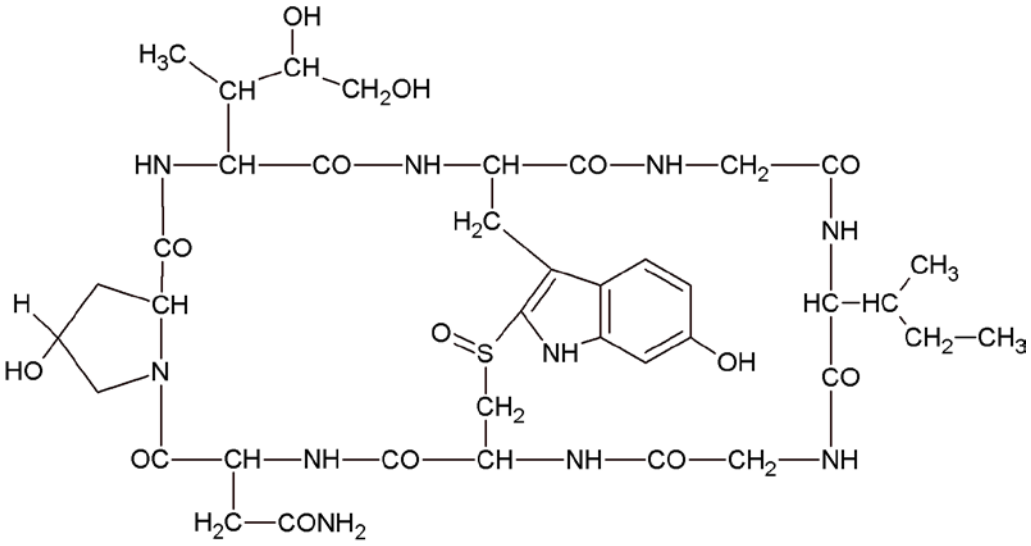


Figure 1
Structure of the basidiomycete toxin alpha-amanitin, a cyclic octapeptide.

1 Fermentation and downstream process for the production of alpha-amanitin - a prerequisite for the development of antibody-amanitin conjugates for tumour therapy

Group Leader: Martin Roth

A general objective in oncology drug discovery and development is to achieve tumour cell eradication, while maintaining at the same time a high level of tolerability to healthy cells and tissues. Antibody-drug conjugates (ADCs) combine the well established principle of targeted antibodies (safety and tolerability) with the effectiveness of potent toxins (anti-tumour efficacy). They consist of a monoclonal antibody conjugated to toxins by chemical linkers. The function of the antibody part of the ADC molecule is 'to guide' the toxin specifically to the target tumour cells, and to allow healthy cells to be left untouched.

Currently approved therapeutic monoclonal antibodies, such as MabThera, Herceptin, Avastin and Erbitux have made anti-cancer therapy more effective, safer and better tolerated than with conventional chemotherapeutics. Maytansinoids, auristatins and others may effectively induce tumour shrinkage and reduce tumour mass in selected tumour types – by acting selectively on dividing tumour cells.

Current opinion however is, that tumour relapse and tumour metastases may be related to "dormant or quiescent tumour cells" that are insensitive to most – if not all - currently approved drugs for the treatment of cancer. This explains the tremendous demand for toxins and coupling techniques that result in new ADCs with entirely new properties, in particularly to meet the challenges in treating tumour metastases and preventing tumour relapses.

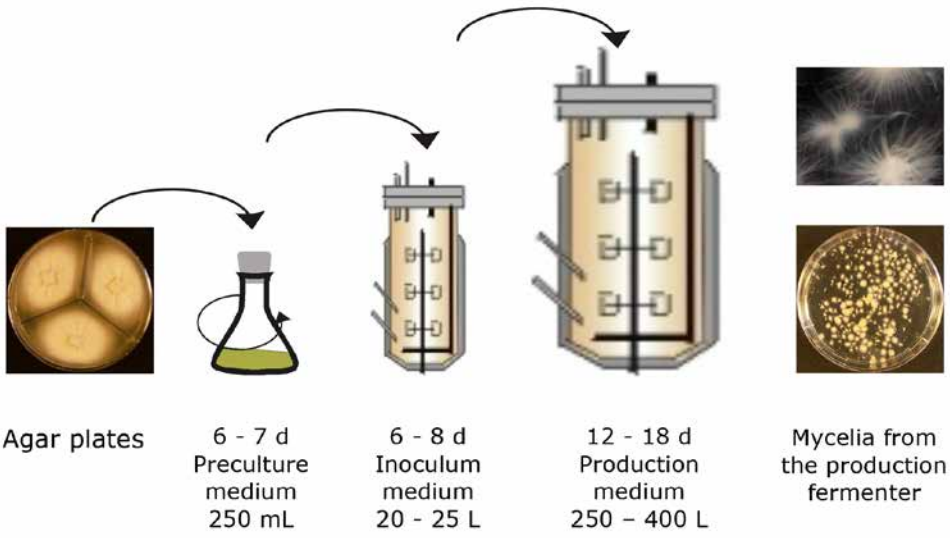


Figure 2
Scheme of the alpha-amanitin fermentation process.

In this environment, the Heidelberg Pharma GmbH (HDP) concentrates on combining the targeting properties of antibodies with the effectiveness of the ultra-potent basidiomycete toxin alpha-amanitin, a specific inhibitor of RNA polymerase II.

Amanitin differs from other toxins used in today's ADC therapeutics by its unique intracellular target and its systemic and proteolytic stability. ADCs with amanitin (AMA-ACs) have the potential to treat multiresistant tumours and 'dormant or quiescent' tumour cells, which are regarded as a main reason for metastasis and resistance.

For the first time Heidelberg Pharma has evaluated an AMA-AC with the monoclonal antibody Herceptin. It was shown in different *in vitro* and *in vivo* models of oncology that the ADC possesses potent antitumoural activity in HER2+ cells and tissues.

The availability of sufficient amounts of alpha-amanitin is a prerequisite for the development of AMA-ACs and clinical trials to test their efficiency. However, up to now neither the chemical synthesis nor a biotechnological production process were known.

It was therefore the aim of a project at HKI funded by the BMBF in the program KMU-innovativ, to develop a GMP-conform biotechnological process to produce alpha-amanitin. Alpha-amanitin is the main component of the amatoxins, bicyclic octapeptides produced by basidiomycetes of the genus *Amanita*, e.g. the Green Deathcap mushroom *Amanita phalloides*. But also in species of other genera as *Lepiota*, *Conocybe* and *Galerina* amatoxins were detected.

Because of their very slow growth under laboratory conditions, mycorrhizal fungi of the genus *Amanita* are not suitable for the development of a fermentation process.

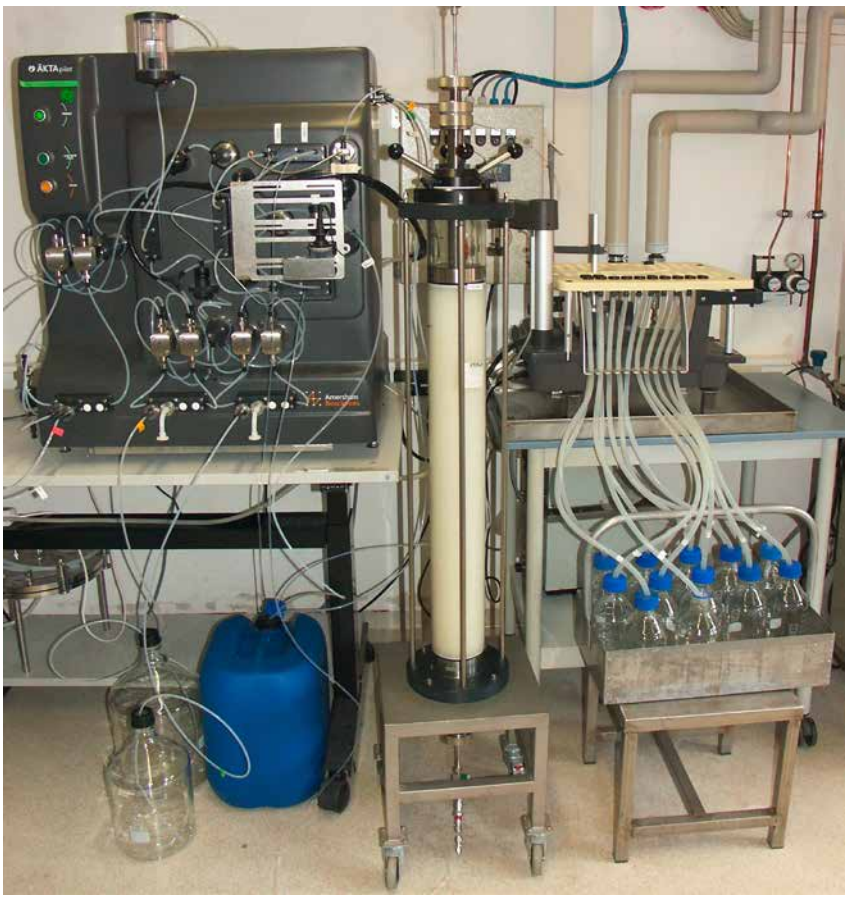


Figure 3
FPLC system (Äkta Pilot) used for separation of amanitins by gel permeation chromatography.

White rot saprophytic basidiomycetes of the genus *Galerina* were known to grow in shake flask cultures. Not only the fruiting bodies but also vegetative mycelia may contain amatoxins.

We selected an amatoxin producing strain of *Galerina marginata* from a variety of isolates tested. Media for precultures and production fermentation were developed in numerous shake flask culture trials.

The fermentation process was then scaled-up via the 30 and 75 litre scale to the 300 litre pilot fermenter. 23 fermentations in the 30 litre scale, 5 in the 75 litre fermenter and 15 in the 300 litre fermenter were performed.

The optimised fermentation process is robust with respect to differing fermenter geometry or inoculum volume. In the fermentation culture an alpha-amanitin yield of 12 - 15 mg/l was reproducibly obtained.

In parallel to the fermentation, a purification process for alpha-amanitin comprising of four chomatography steps was developed. For the improvement of the downstream process more than 4,000 litre culture broth were used. The process allows for production of alpha-amanitin with a purity >90 % at a yield of 40 % in relation to the concentration in the fermentation culture. The purification process can also be applied for production of beta- and gamma-amanitin.

The complete production process is conform to GMP standards. The transfer of the production process to a GMP certified industrial partner of Heidelberg Pharma is in preparation in order to provide the toxin for the production of AMA-ACs for clinical trials.

During the project time purified alpha-amanitin was delivered to the project partner Heidelberg Pharma for the development work with antibody-amanitin conjugates.

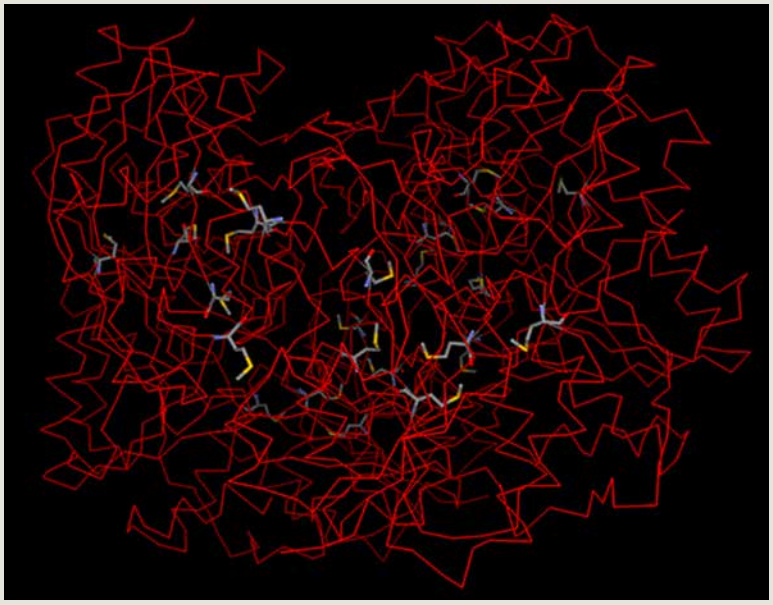


Figure 4
Therapeutic protein structure model with possible misincorporation sites (highlighted grey) of non-canonical amino acids.

In cooperation with Heidelberg Pharma GmbH. Funded by Bundesministerium für Bildung und Forschung, FKZ 0315369B (2008-2011)

2 Influence of stress responses on the amino acid metabolism during high cell density fermentation of recombinant *Escherichia coli*

Group Members: Michael Biermann, Guido Seidel, Peter Neubauer, Uwe Horn

It is known that misincorporation of rare amino acids into recombinant proteins can occur during High Cell Density Fermentation (HCDF) of *Escherichia coli*. For therapeutically relevant proteins the ratio of misincorporation should be as low as possible, as all by-products require special effort during clinical studies and intensive analytical characterisation to obtain a biologics license application. Our goal is to investigate physiological conditions which lead to the synthesis of these non-canonical amino acids and thereby find possible strategies to circumvent their unwanted misincorporation.

Impact of trace element composition in high cell density media on the synthesis of non-canonical amino acids in recombinant *Escherichia coli* fermentations

Escherichia coli is one of the major microbial workhorses used in modern recombinant protein biotechnology. It is commonly cultivated in an aerobic glucose limited growth mode primarily aiming to achieve low by-product formation and high biomass concentrations. The media used in high cell density fermentation processes do normally not contain the essential cofactors of the formate hydrogen lyase complex which is responsible for the disproportionation of formate to carbon dioxide and dihydrogen under anaerobic conditions. The latter situation typically oc-

Figure 5
Fermentation set-up for the parallel high cell density cultivation of recombinant *Escherichia coli*.

curs in large scale processes where insufficient mixing of the cultivation medium leads to gradients in dissolved oxygen tension and glucose concentration especially in the substrate feeding zone. Since the synthesis of non-canonical amino is shown to be induced by anaerobic conditions and pyruvate overflow a connection to the trace element composition is likely.

We performed different sets of fed-batch cultivations (Figure 5) of *E. coli* expressing the VHH antibody domain B10 under oxygen and glucose stress conditions. The analysis of the amino acid metabolism shows a lower synthesis of rare amino acids under stress in the fermentation medium with the addition of certain trace elements. Our data indicate that these elements should be added generally to fermentation media used for the production of sensitive recombinant proteins to prevent misincorporation of non-canonical amino acids.

Analysing strain dependent differences in the amino acid metabolism of *Escherichia coli*

Here we address the question of the rare amino acid synthesis by analysis of recombinant K12- and B-strain dependent differences of glucose metabolism related stress response recombinant *Escherichia coli* fermentations. By using different stress inducing fed-batch fermentation set-ups, precise U-HPLC measurement of involved metabolites and the expression analysis of the target protein we show that recombinant *E. coli* B-strains produce lower levels of non-proteinogenic amino acids under stress conditions and respond via the overflow synthesis of other branched chain amino acids in comparison to *E. coli* K12. Our data indicate that *E. coli* B is the favourable strain variant regarding the unwanted synthesis of rare amino acid species in fermentation processes.

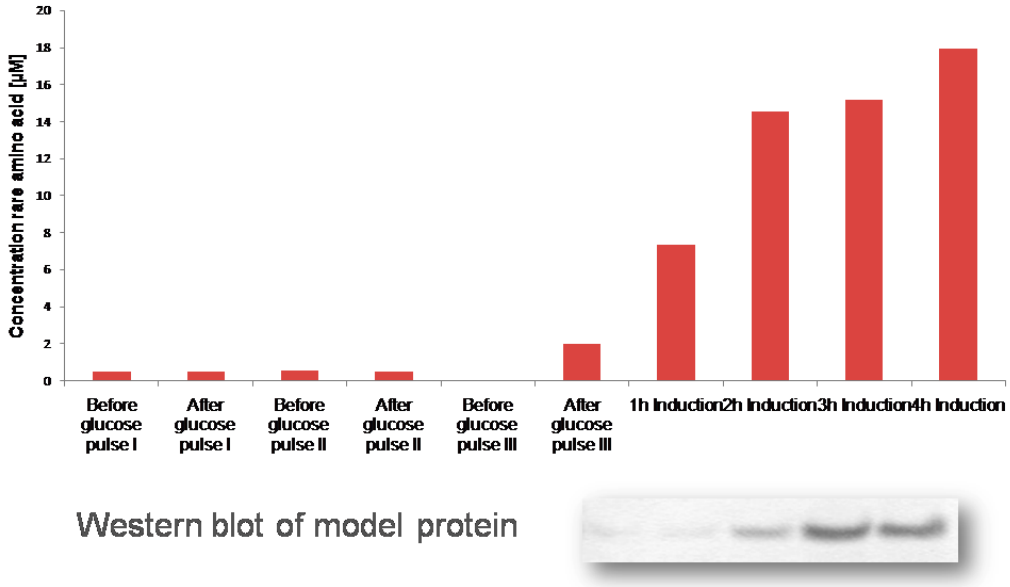


Figure 6
Amino acid profile and expression of model protein (t_0, before induction, t_1-t_4, 4h induction phase) during stress fermentation.

These results enable a broad range of further studies on the molecular mechanism of overflow stress response. A closer look towards various connections of the central carbon and amino acid metabolism reveals possible strategies in the prevention of misincorporation of non-proteinogenic amino acids into recombinant proteins.

In cooperation with Wacker Biotech GmbH and Technical University Berlin.

3 Studying biofilm formation on biomaterials

Group members: Claudia Lüdecke, Martin Roth, Jörg Bossert (Institute of Materials Science and Technology, Friedrich Schiller University Jena) and Klaus D. Jandt (Institute of Materials Science and Technology, Friedrich Schiller University Jena)

Biomaterials for implant purposes are increasingly applied in modern medicine e. g. to restore human body functions. Infections associated with these implants are primarily initiated by the adhesion of microorganisms and subsequent biofilm formation on the biomaterials surface. The chemical and structural properties of the implant surface significantly influence the interaction between the biomaterials surface and the biological system, and thus determine the potential antimicrobial properties of the biomaterial surfaces. In the last two decades, numerous

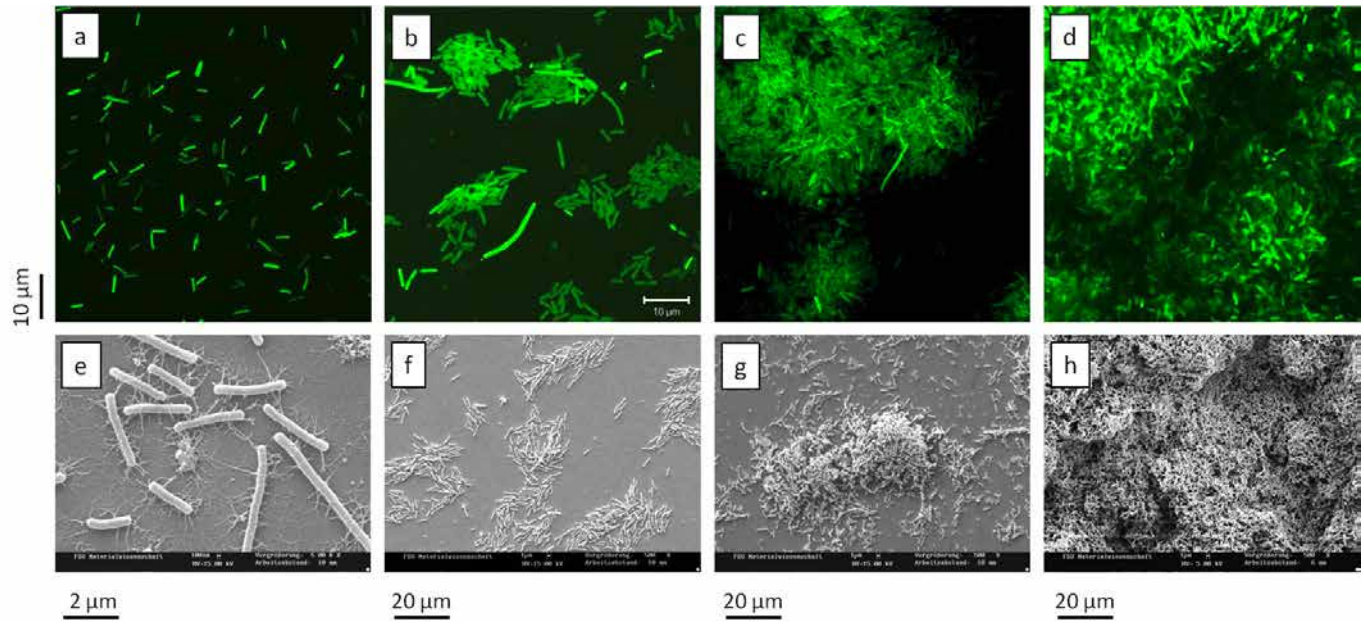


Figure 7
Biofilm formation on titanium surfaces: CLSM images (a-d) and SEM images (e-h) of the typical four-stages biofilm formation of *Escherichia coli* including the randomly initial adhesion after 24 hours (a, e), micro colony formation after 48 hours (b, f), growth and increase of the height after 3 days (c, g), and maturation of the biofilm after 6 days (d, h).

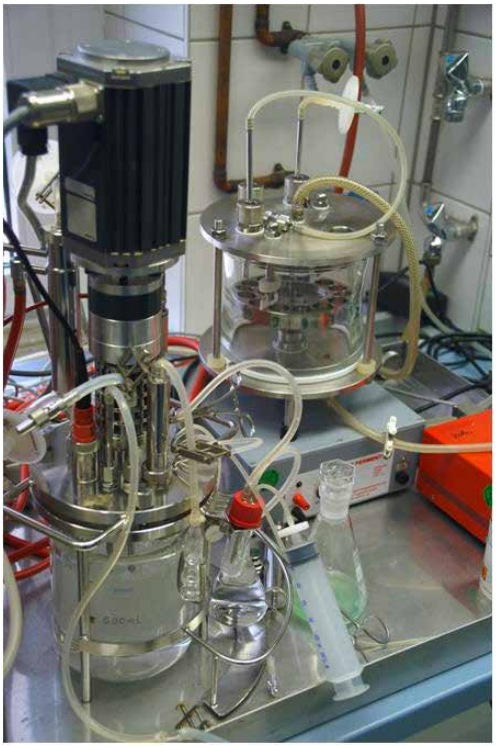
investigations were made about the influence of material surface properties on the adhesion of microorganisms. However, these studies are hard to compare since there are no standard tests for biofilm formation and characterisation.

The aim of this project was, therefore, to design and evaluate a standard *in vitro* testing device allowing the investigation of microbial adhesion and subsequent biofilm formation on various biomaterials in relation to their surface properties.

To realise the test device, a continuous culture (chemostat) was coupled to a rotating disc biofilm reactor. A continuous culture is a flow system, where nutrients are supplied at a constant rate to the growing culture in a fermenter, whereas the total culture volume remains constant. In that way the bacterial population reaches a "steady state" of cell growth, in which the total number of bacteria and the rate of cell division remain constant. The biofilm reactor for biofilm cultivation, connected to the continuous culture system, consists of a glass vessel housing a stainless steel rotating disc with sample pans adapted to various biomaterials. The turn table rotates under PTFE scrapers that homogeneously spread the incoming inoculum (continuous culture) on all pans with the biomaterials samples.

This set up allows the biofilm cultivation under static conditions with a continuous exchange of the inoculum/bacterial suspension. In that way the bacteria are always in the same physiological state and conditions for biofilm cultivation are highly reproducible during the experiment and also between different experimental runs.

For the evaluation of the *in vitro* test system, the model organism *Escherichia coli* constitutively producing the green fluorescent



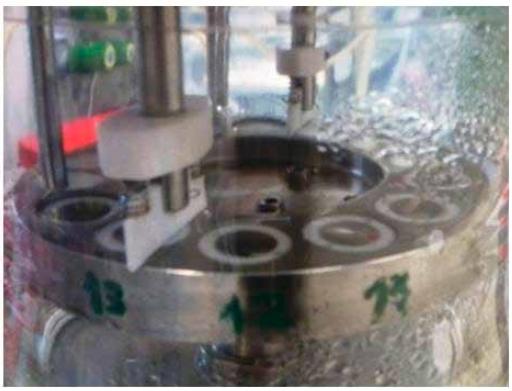


Figure 8
Set up of the biofilm testing device including the continuous cultivation of the model organism and the biofilm reactor (left) and the control unit for monitoring and adjusting the cultivation conditions within the fermenter (top right). The biofilm reactor consists of a rotating disc with 14 wells containing sample holders with titanium samples (bottom right). The samples are inoculated drop wise with the continuous culture while the disc is slowly rotating.

protein (GFP) was cultivated on physical vapour deposited titanium thin films. Titanium surfaces were characterised by atomic force microscopy (AFM). The biofilm development was investigated with scanning electron microscopy (SEM), confocal laser scanning microscopy (CLSM) and a subsequent image analysis.

During three independent evaluation runs of the testing device the biofilms showed statistically identical growth concerning their height, substrate coverage and biovolume, with a typical four-stages-development including initial bacterial adhesion, micro colony formation and early and late maturation.

Our results show that the new standard biofilm testing device is suitable (i) to cultivate statistically reproducible biofilms, (ii) to characterise biofilm formation on biomaterial surfaces, including formation kinetics, and thus, (iii) to examine the influence of biomaterials and biomaterial surface properties on the biofilm formation.

In cooperation with Institute of Materials Science and Technology, Chair in Materials Science (CMS), the Friedrich Schiller University Jena.

Funded by Jena School for Microbial Communication (JSMC), TMBWK in the “ProExzellenz” program “MikroInter”.

4 Hunting for natural products within droplets: Screening for antibiotics using microfluidics

Group members: Emerson Zang, Martin Rudolph, Karin Martin, Martin Roth

Due to the increasing emergence of multiresistant pathogens, there is an ongoing need for new antibiotics. Most currently available

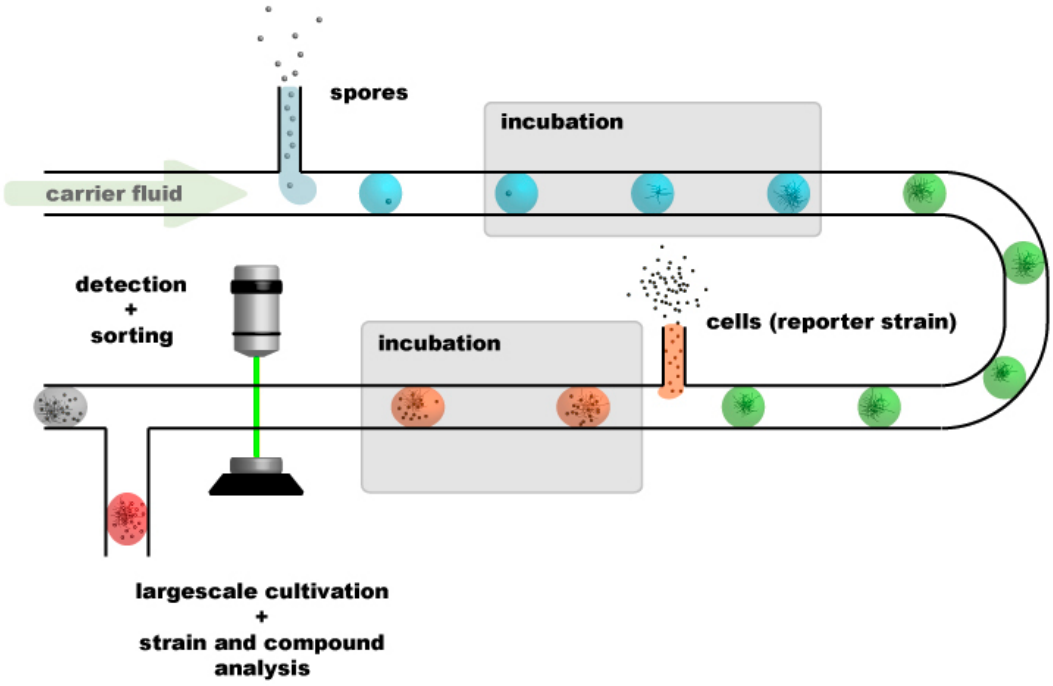


Figure 9
Whole-Cell-Screening for novel antibiotics from actinobacteria in a microfluidic system.

References

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Davies J, Spiegelman GB, Yim G (2006) The world of subinhibitory antibiotic concentrations. *Current opinion in microbiology* 9, 445-453.

antibiotics are derived from assertive and ubiquitous actinobacteria (e.g. *Streptomyces, Actinomadura, etc.*), which are persistently rediscovered if standard screening techniques are applied. Droplet-based microfluidics provide extremely powerful means for the high-throughput detection of antimicrobial activity in complex biological samples. We developed a monolithic whole-cell-assay chip device for antimicrobial screening of droplet-enclosed actinomycete cultures. It allows for generation of pure actinomycete droplet-cultures from a spore suspension, addition of a reporter strain and subsequent optical readout of antibiotic activity (Figure 9).

As a proof-of-principle, we generated on-chip a droplet series with increasing concentration of the streptothricin antibiotic nourseothricin (Ntc) and added a red fluorescent *E. coli* reporter strain. After 2d of incubation at 37 °C, droplets with low Ntc-concentration were highly fluorescent, while droplets at higher concentrations revealed nearly no fluorescence (Figure 10).

The transition from non-inhibitory to inhibitory conditions enabled the determination of a minimal inhibitory concentration (MIC) of 4 µg/ml, which corresponds perfectly with values found in corresponding microtiter plate experiments. According to Zhang *et al.* (1999), the Z'-factor of 0.59 calculated from positive and negative controls verifies this concept as an „excellent assay". In order to mimic biological complexity as expected in a real screening, in a follow-up experiment the Ntc-solution was replaced by culture supernatant of the Ntc-producing bacterium *Streptomyces noursei* (Figure 11). Fluorescein was added prior to droplet generation to be able to track the supernatant concentration in each droplet by green fluorescence (green curve). Beside non-fluorescent droplets (in-

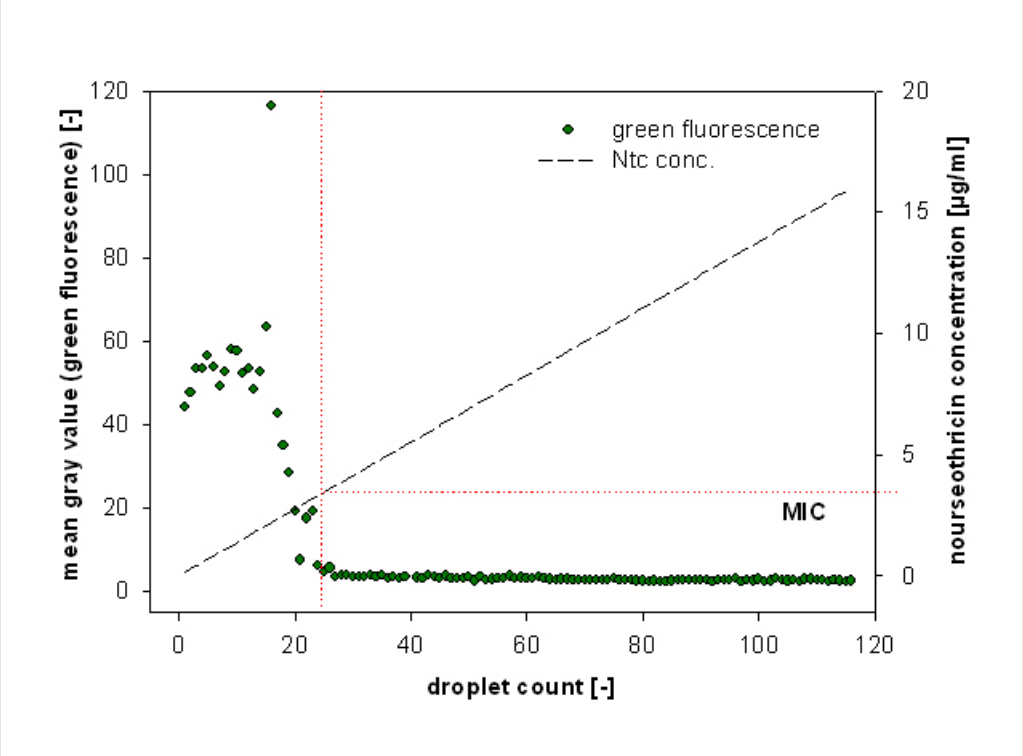

Figure 10
Determination of minimal inhibition concentration for nourseothricin and an *E. coli* reporter strain in a monolithic, microfluidic chip device.

hibition) and droplets with normal fluorescence (no inhibition), a third droplet species with manifold enhanced fluorescence was detected – preferably at the transition from non-inhibitory to inhibitory conditions. It can be assumed that the enhanced fluorescence indicates elevated metabolic activity and protein synthesis, induced by subinhibitory antibiotic concentrations („hormesis"), as already described by Davies *et al.* (2006). The demonstrated tools now meet all requirements to screen in the microfluidic system for novel antimicrobial substances.

5 Production of microbial biomass and natural products for research projects of HKI groups and external academic partners

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

A large number of microbial products from different microorganisms were produced for projects of research groups at HKI. 564 fermentations at the 0.5 to 5 litre labscale and 55 fermentations at the 30 to 300 litre pilot scale were performed within 2010-2011 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

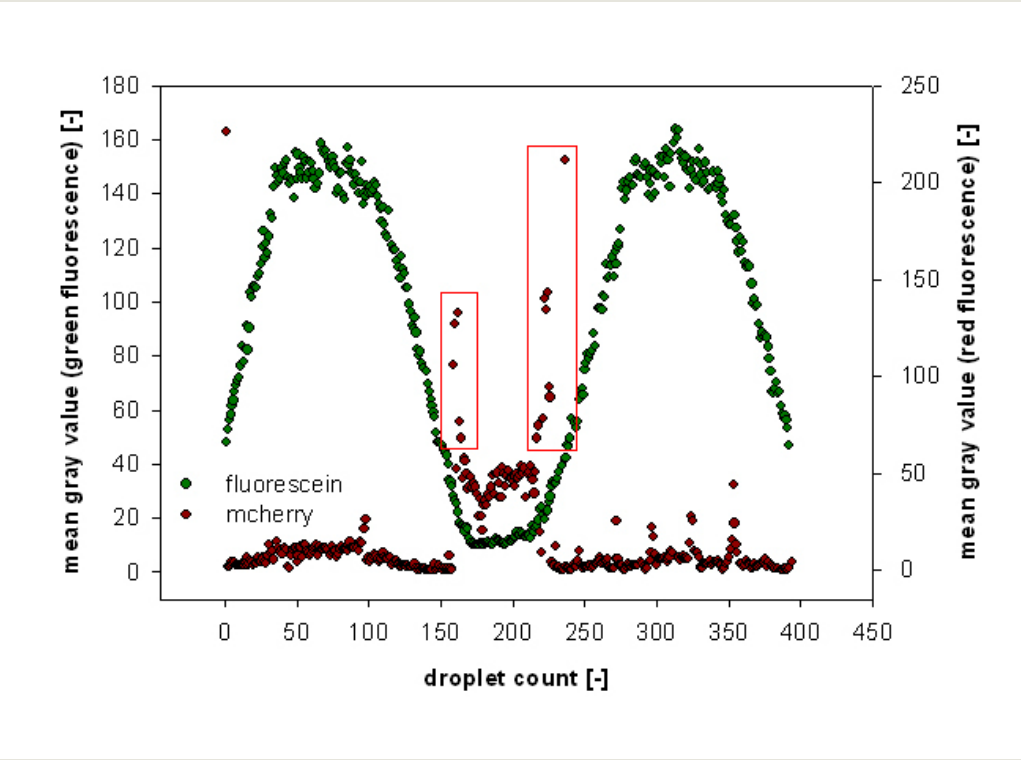

Figure 11
Inhibition of red-flourescent *E. coli* by *S. noursei* culture supernatant. The red boxes indicate droplets with enhanced reporter activity due to subinhibitory antibiotic concentrations.

and freeze drying of the extracts. In 2010-2011 products from about 3,500 litre fermentation broth were prepared.

Collaborations with research groups at the HKI and with external academic partners

Project
Screening for Bioactive Natural Products (HKI, Dept. Biomolecular Chemistry)
Activities at Bio Pilot Plant
Pilot scale fermentations (300 L) of microorganisms (actinomycetes from unusual habitats, endophytes from mangrove plants, rare actinomycetes from heavy-metal contaminated habitats) to isolate and produce new bioactive natural products.
Downstream processing, especially preparative HPLC, to isolate new bioactive substances.

Project
Discovery of new drugs and metabolites (HKI, Junior Research Group Secondary Metabolism of Predatory Bacteria)
Activities at Bio Pilot Plant
Pilot scale fermentation (30-300 L) of predatory and phytopathogenic bacteria, and downstream processing to isolate new natural products.

Project
Genome mining in clostridia for natural product discovery (HKI, Dept. Biomolecular Chemistry)
Activities at Pilot Plant
440 anaerobic cultivations of *Clostridium* species in lab-scale fermenters (0.5-5 L) were performed for the isolation of novel natural products.

Project
Screening for Bioactive Natural Products (HKI, Dept. Biomolecular Chemistry)

Project
Investigation of secondary metabolite biosyntheses in *Burkholderia* species (HKI, Dept. Biomolecular Chemistry)
Activities at Pilot Plant
Pilot scale fermentation of *Burkholderia rhizoxinica* and *B. thailandensis* strains and downstream processing to isolate secondary metabolites.

Project
Biosynthetic Mechanisms and Processing Lines (HKI, Dept. Biomolecular Chemistry)
Activities at Bio Pilot Plant
Laboratory and pilot scale fermentation (5 - 30 L) of *Aspergillus fumigatus* mutants and *Burkholderia* strains.

Project
Use of heavy metal resistant streptomycetes in bioremediation (Friedrich Schiller University Jena, Institute of Microbiology, Microbial Phytopathology)
Activities at Bio Pilot Plant
Pilot scale fermentation (30-300 L) of heavy metal resistant *Streptomyces* strains and preparation of biomass for bioremediation studies at the former uranium mining site in Eastern Thuringia.

Project
Investigation of covalently linked cell wall proteins by *Candida albicans* Sap-proteins (HKI, Dept. Microbial Pathogenicity Mechanisms)
Activities at Pilot Plant
Laboratory scale fermentation of recombinant *Pichia pastoris* and downstream processing. Purification of *Candida albicans* Sap-proteins.

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External funding

Bundesministerium für Bildung und Forschung
Synthetische und biotechnologische Herstellung von Amanitin
Project: Biotechnologische Herstellung
Martin Roth

Bundesministerium für Bildung und Forschung
Zentrales Innovationsprogramm Mittelstand (ZIM)
Kameliden Antikörper basierter ELISA zur simultanen Bestimmung relevanter Mykotoxine
Hans Krügel, Uwe Horn

Bundesministerium für Bildung und Forschung
DiNaMid – Genom-basierte Findung neuer antimikrobieller Naturstoffe in mikrofluidischen Chips; Identifizierung neuer Stoffwechselwege und Naturstoff-Isolierung
Markus Nett, Martin Roth

Deutsche Forschungsgemeinschaft
Jena School for Microbial Communication
Project: Development of a procedure to avoid misincooperation of rare amino acids into therapeutic proteins during High Cell Density Fermentation
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Kusebauch B, Busch B, Scherlach K, **Roth M**, Hertweck C (2010) Functionally distinct modules operate two consecutive alpha,beta->beta,gamma double-bond shifts in the rhizoxin polyketide assembly line. *Angew Chem Int Ed* 49, 1460-1464.

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International Leibniz Research School for Microbial and Biomolecular Interactions

International Leibniz Research School for Microbial and Biomolecular Interactions

The *"International Leibniz Research School for Microbial and Biomolecular Interactions"* (ILRS Jena) was established in 2005. It is lead by the Leibniz Institute for Natural Product Research and Infection Biology – Hans Knöll Institute – in collaboration with the Friedrich Schiller University Jena (FSU) and the Max Planck Institute for Chemical Ecology (MPI CE). Initially financed by the competitively granted means of the Joint Initiative for Research and Innovation, the graduate school has been included in the HKI budget since 2008.

The close ties of the HKI with the MPI CE and with various institutes of the university provide excellent research conditions in the field of microbial and biomolecular interactions. Since 2006, ILRS forms part of the *Jena School for Microbial Communication*, a graduate school funded by the Excellence Initiative of the German Research Foundation. The Ph.D. students benefit from the scientific exchange and extended choice of courses offered by the close network with other graduate schools in Jena and the Jena Graduate Academy. Approximately half of the students in ILRS come from abroad, extending Jena's worldwide network and enhancing its international visibility.

The first two ILRS graduate students successfully defended their Ph.D. theses in the year

Die *„International Leibniz Research School for Microbial and Biomolecular Interactions"* (ILRS Jena) wurde nach Bewilligung des Antrages im Pakt für Forschung und Innovation der Leibniz-Gemeinschaft im Herbst 2005 ins Leben gerufen. Sie wird federführend vom Leibniz-Institut für Naturstoff-Forschung und Infektionsbiologie – Hans-Knöll-Institut (HKI) – gemeinsam mit der Friedrich-Schiller-Universität Jena (FSU) und dem Max Planck Institut für chemische Ökologie (MPI CE) organisiert. Seit 2008 ist die Graduiertenschule im Haushalt des HKI verstetigt.

Die enge Verflechtung des HKI mit dem MPI CE und den Instituten der Friedrich-Schiller-Universität zum Thema mikrobielle und biomolekulare Interaktion bietet eine hervorragende Chance, herausragende wissenschaftliche Leistungen zu erzielen. Die ILRS ist seit 2006 Mitglied der mit Mitteln der Exzellenz-Initiative geförderten *Jena School for Microbial Communication* (JSMC). Die Doktoranden profitieren vom wissenschaftlichen Austausch und erweiterten Kursangebot durch die enge Vernetzung der ILRS mit anderen Graduiertenschulen und der Jenaer Graduiertenakademie. Etwa die Hälfte der Doktoranden der ILRS kommt aus dem Ausland und fördert so die internationale Vernetzung und erhöht nachhaltig die internationale Attraktivität Jenas.

Die ersten beiden Doktoranden der ILRS schlossen bereits im Jahr 2009 ihre Dissertation mit großem Erfolg ab. In den Jahren 2010

2009. In the following two years, a further 14 students graduated from ILRS. After their graduation, they moved on to responsible jobs in industry or academia. The second group of 20 Ph.D. students started during 2009 and 2010, the first among them will defend their theses in early 2012. The third round of recruitment was initiated at the end of 2011. Approximately 350 students from 37 countries applied for the offered ILRS projects.

Prof. Peter Zipfel has been the Speaker of ILRS since 2008; the other members of the Steering Committee are Prof. Ian T. Baldwin, Prof. Gabriele Diekert and Prof. Bernhard Hube.

Scientific Topics

In their natural habitat, microorganisms do not occur in isolation but live in close association with other organisms of different species and developmental stages. Their interactions can take many forms, ranging from mutual symbiosis to parasitic interactions. How these complex, multi-organismic networks are regulated by microbial communication is still poorly understood. The ILRS Ph.D. projects therefore aim to gain insight into the microbial and biomolecular interactions underlying the communication processes among microorganisms in diverse habitats with interdisciplinary approaches, combining methods of microbiology, natural

und 2011 folgten weitere 14 Doktoranden. Im Anschluss an ihre Promotion nahmen sie verantwortungsvolle Tätigkeiten in der akademischen oder industriellen Forschung auf. Die zweite Gruppe von 20 Promovierenden begann im Laufe der Jahre 2009 und 2010; die ersten von ihnen werden voraussichtlich Anfang 2012 ihre Dissertation verteidigen. Ende 2011 startete die dritte Ausschreibung bei der sich ca. 350 Studenten aus 37 Nationen auf die ILRS-Projekte bewarben.

Prof. Peter F. Zipfel ist seit 2008 Sprecher der ILRS, die stellvertretenden Sprecher sind Prof. Ian T. Baldwin, Prof. Gabriele Diekert und Prof. Bernhard Hube.

Wissenschaftliche Themenschwerpunkte

Mikroorganismen kommen in der Natur in der Regel nicht als Einzelzellen vor, sondern leben in den verschiedensten Habitaten im Verbund mit anderen Organismen, z. B. in mutualistischen Symbiosen oder aber in parasitären Interaktionen. In der Umwelt stehen Mikroorganismen in Wechselwirkung mit vielen anderen Mikroorganismen (Bakterien/Bakterien, Bakterien/Pilze, Pilze/Pilze) und auch mit höheren Organismen wie Pflanzen und Tieren. Die tragenden Kommunikationsmechanismen dieser Interaktionen sind weitgehend unverstanden. Die Forschungsprojekte im Rahmen der ILRS versuchen daher, die zugrunde liegenden mikrobiellen und biomolekularen Interaktionen

product chemistry, chemical ecology, bioinformatics and systems biology.

Structured PhD Training

The implementation of a curriculum during the course of the Ph.D. program is an integral part of the structured Ph.D. training. This curriculum consists of the following:

a) An accompanying series of colloquia and seminars
b) Each ILRS faculty member teaches advanced methods from his field of expertise in a practical course open to all members of the ILRS
c) Special soft skill courses such as "Scientific Writing", "Poster and Oral Presentations", "Research Funding", as well as Public Relations Activities and German and English Language courses are also offered.

In addition, there are regular group seminars and an annual international symposium.

All means of education follow a program of study specifically developed for ILRS. Thus, an efficient qualification of the junior researchers within a tight time frame is ensured.

mit Methoden der Mikrobiologie, Naturstoffchemie, chemischen Ökologie, Bioinformatik und Systembiologie zu entschlüsseln.

Strukturierte Doktorandenausbildung und Lehre

Wesentlicher Bestandteil der strukturierten Doktorandenausbildung ist die Implementierung von Lehrveranstaltungen in die Dissertationszeit. Diese werden in drei Formen angeboten:

a) Im Rahmen der ILRS wird eine studienbegleitende Kolloquien- und Seminarreihe abgehalten.
b) Jeder an der ILRS beteiligten Projektleiter bietet ein Methodenpraktikum auf dem Gebiet seiner Expertise an, welches von allen Mitgliedern der ILRS besucht werden kann.
c) Spezielle soft skill-Kurse, wie *„Scientific Writing"*, *„Poster and Oral Presentations"*, *„Research Funding"*, sowie *„Public Relations Activities"* und Sprachkurse in Deutsch und Englisch werden angeboten.

Außerdem finden regelmäßig Gruppenseminare und jährlich ein internationales Symposium statt. Alle ausbildungsrelevanten Maßnahmen werden von den Doktoranden entsprechend einem für die ILRS entwickelten Program of Study absolviert. Dies sichert eine effiziente Qualifikation des wissenschaftlichen Nachwuchses in einem stringenten Zeitschema.

ILRS Faculty Member	Title of Ph.D. Project	Ph.D. Student
Prof. Ian T. Baldwin	Characterizing and analyzing the ecological consequences of the plant - endophyte interactions of *Solanum nigrum* and *Nicotiana attenuata*	Hoang Hoa Long
Prof. Ian T. Baldwin	Microbial interactions relevant for the fitness of *Nicotiana attenuata* in the native environment	Arne Weinhold
Prof. Wilhelm Boland	Induction of metabolic shifts in Streptomyces	Anne Behrend
Prof. Wilhelm Boland	Isolation, purification, and structural elucidation of active compounds from the tissue of insect	Huijuan Guo
Prof. Axel A. Brakhage	Regulation of cell wall integrity signalling by mitogen-activated protein kinase MpkA in *Aspergillus fumigatus*	Radhika Jain
Prof. Axel A. Brakhage	Molecular mechanisms of the interaction between *Aspergillus fumigatus* and alveolar macrophages	Andreas Thywissen
Prof. Axel A. Brakhage/ Prof. Johannes Wöstemeyer	Secretome analysis/ pathogenicity mechanisms of *Arthroderma benhamiae*	Christoph Heddergott
Dr. Matthias Brock/ Prof. Bernhard Hube	Host pathogen interactions of human-pathogenic yeast	Shruthi Ramachandra
Dr. Matthias Brock/ Prof. Bernhard Hube	Lysine biosynthesis in *Aspergillus fumigatus*	Felicitas Schöbel
Dr. Ralf A. Claus/ Prof. Konrad Reinhart	Ceramide formation in host response to bacterial and fungal infection and development of organ failure	Nayla Jbeily
Prof. Gabriele Diekert	Expression control and biosynthesis of dehalogenating enzymes from anaerobic soil bacteria in response to the interaction with aerobic halogenating fungi	Anita Mac Nelly
Prof. Gabriele Diekert	Studies on the microbial halogen cycle: reactions of fungal peroxidases and bacterial reductive dehalogenases	Lidan Ye
Dr. Reinhard Guthke	Integration of transcriptome and proteome data from human-pathogenic fungi	Daniela Albrecht
Dr. Reinhard Guthke	Prediction of gene regulatory networks involved in the differentiation, secondary metabolism and cross talk of *Aspergillus nidulans*	Fabian Horn

Continuation →

ILRS Faculty Member	Title of Ph.D. Project	Ph.D. Student
Dr. Reinhard Guthke	Error correction for the integration of proteome and transcriptome data	Christian Hummert
Dr. Reinhard Guthke	Integrated genome-wide data analysis by ensemble learning methods to understand infection processes	Sebastian Müller
Prof. Christian Hertweck	Genome mining of Gram-positive bacteria for secondary metabolites	Swantje Behnken
Prof. Christian Hertweck	The genome of the endofungal bacterium *Burkholderia rhizoxinica*	Gerald Lackner
Dr. Uwe Horn	Regulation of polyketide synthase gene clusters in *Aspergillus nidulans*	Alexander Funk
Dr. Uwe Horn	Characterisation of silent secondary metabolite gene clusters in the filamentous fungi: *Aspergillus nidulans*	Anindita Sarkar
Dr. Uwe Horn/ Prof. Dirk Hoffmeister	Natural products from an unidentified homobasidiomycete which control wood- deteriorating microorganisms	Daniel Schwenk
Prof. Bernhard Hube	Identification and characterisation of infection-associated genes in *Candida albicans*	Francois Mayer
Dr. Olaf Kniemeyer	The hypoxic (low-oxygen) response of the pathogenic mould *Aspergillus fumigatus* and its relevance to pathogenicity	Kristin Kroll
Prof. Erika Kothe	Expression and localisation of G-protein coupled pheromone receptor Bar2 in the basidiomycete *Schizophyllum commune*	Susann Erdmann (nee Jezewski)
Prof. Erika Kothe	Pheromone response in the model basidiomycete *Schizophyllum commune*	Dominik Senftleben
Prof. Maria Mittag	An insertional mutagenesis approach to understand temperature entrainment of the circadian clock in *Chlamydomonas reinhardtii*	Karthik Mohan
Prof. Maria Mittag	Functional characterisation of molecular components of the circadian clock of the green alga *Chlamydomonas reinhardtii*	Stefanie Seitz (nee Kiaulehn)
Prof. Johannes Norgauer	Function of phosphatidylinositol-3-kinase-·amma and SH2-containing inositol-5-phosphatase-1 in innate immunity	Krisztina Truta-Feles

ILRS Faculty Member	Title of Ph.D. Project	Ph.D. Student
Prof. Georg Pohnert	Unravelling the (bio)chemical processes involved in phytoplankton-virus interactions	Michaela Mauß
Prof. Georg Pohnert	Regulation of the microbial community by the green alga *Dictyosphaeria ocellata*	Jennifer Sneed
Prof. Hans Peter Saluz	Acute and chronic *Aspergillus* infection in mice studied by PET-CT and comparative gene expression	Shayista Amin
Prof. Hans Peter Saluz	Molecular study of apoptotic processes in *Aspergillus ssp.* and the influence of *Aspergillus fumigatus* on apoptosis in host immune effector cells	Katrin Volling
Dr. Christine Skerka	Complement evasion of human pathogenic microorganisms	Tina Enghardt
Prof. Eberhardt Straube	Modulation of host cell activation and apoptosis by *Chlamydia trachomatis*	Hangxing Yu
Prof. Günter Theißen	Interactions and regulatory networking of MADS-domain Transcription factors in model plants. (*Arabidopsis thaliana*)	Khushboo Jetha
Prof. Günter Theißen	Reconstructing the interaction network of reproductive homeotic proteins in a gymnosperm	YongQiang Wang
Prof. Gunter Wolf	Complement regulation in kidney cells	Isabell Kopka
Prof. Peter F. Zipfel	The role of complement in autoimmune disease	Qian Chen
Prof. Peter F. Zipfel	The role of CFHR proteins in human autoimmune diseases	Hannes Eberhardt
Prof. Peter F. Zipfel	*Candida albicans*-host interaction, the many faces of candida Pra1	Shanshan Luo
Prof. Peter F. Zipfel	Functional characterisations of variant forms of the human complement regulator CFHR1	Selina Stippa
Prof. Peter F. Zipfel/ Prof. Johannes Wöstemeyer	Complement escape mechanisms of *Streptococcus pneumoniae*	Sarbani Sarkar

Cross-sectional Unit
Jena Microbial Resource Collection

Cross-sectional Unit Jena Microbial Resource Collection

The Jena Microbial Resource Collection (JMRC) was established in October 2010 by combin the microbial strain collections of the HKI and the Friedrich Schiller University. As a cross-sectional unit within HKI, it functions as a continuation of the Internal Product Line (IPL) and offers scientific services concerning the collection, curation, storage and global exchange of microbial resources (microorganisms, natural products, testing capacities). The JMRC curates a collection of approx. 50,000 microorganisms (15,000 fungi and 35,000 bacteria). The JMRC is also responsible for a library of natural products, natural product derivatives and synthetics. These collections are used both for research within HKI and for international exchange of material and data within cooperations and collaborative projects. In addition, the JMRC is in charge of coordinating the complete biological profiling of new active compounds in order to prepare the pre-clinical stage of drug development and develops assays to test the antiinfective activity of substances.

INTRODUCTION | EINLEITUNG

Head:
PD Dr. Kerstin Voigt

Die Jena Microbial Resource Collection (JMRC) entstand im Oktober 2010 durch Vereinigung der Mikroorganismen-Sammlungen des HKI und der Friedrich-Schiller-Universität Jena. Als Querschnittseinrichtung innerhalb des HKI führt sie die Arbeit der Durchgehenden Bearbeitungslinie (DBL) fort und bietet wissenschaftliche Dienstleistungen rund um die Sammlung, Verwahrung, Pflege und den weltweiten Austausch von mikrobiologischen Ressourcen (Mikroorganismen, Naturstoffe, Testkapazitäten) an. Die JMRC kuratiert eine Sammlung von ca. 50.000 Mikroorganismen (15.000 Pilze und 35.000 Bakterien). Außerdem betreut die JMRC eine Bibliothek mit Naturstoffen, Naturstoffderivaten und Synthetika. Diese Kollektionen dienen sowohl Forschungsprojekten innerhalb des HKI als auch dem weltweiten Material- und Datenaustausch im Rahmen von Kooperationen und Verbundvorhaben. Die JMRC koordiniert des Weiteren die gesamte biologische Profilierung neuer Wirkstoffe zur Vorbereitung der präklinischen Phase der Medikamentenentwicklung und entwickelt neue Assays zur Prüfung antiinfektiver Aktivitäten. Diesen

Upon request, this service is also offered for large research consortia or research projects for academic or industry partners.

Research with the JMRC focuses on the pathogenesis and on host-pathogen interactions of Zygomycetes within the Department Molecular and Applied Microbiology.

Service bietet die JMRC großen Forschungsverbünden und auf Anfrage in Einzelprojekten für akademische und Industriepartner an.

Im Rahmen ihrer Forschungstatigkeiten widmen sich die Mitglieder der JMRC innerhalb der Abteilung Molekulare und Angewandte Mikrobiologie der Pathogenese und der Wirt-Pathogen-Interaktion von Zygomyceten.

Appendix

Appendix

Peer Reviewed Articles 2010
Originalarbeiten 2010

Department Biomolecular Chemistry

Abdou R, **Scherlach K**, Dahse HM, **Sattler I**, **Hertweck C** (2010) Botryorhodines A-D, antifungal and cytotoxic depsidones from *Botryosphaeria rhodina*, an endophyte of the medicinal plant *Bidens pilosa*. *Phytochemistry* 71, 110-116.

Bergmann S*, Funk AN*, **Scherlach K***, Schroeckh V, Shelest E, Horn U, **Hertweck C**, Brakhage AA (2010) Activation of a silent fungal polyketide biosynthesis pathway through regulatory cross talk with a cryptic nonribosomal peptide synthetase gene cluster. *Appl Environ Microbiol* 76, 8143-8149. * equal contribution

Bruns S, Seidler M, Albrecht D, Salvenmoser S, **Remme N**, **Hertweck C**, Brakhage AA, Kniemeyer O, Müller FM (2010) Functional genomic profiling of *Aspergillus fumigatus* biofilm reveals enhanced production of the mycotoxin gliotoxin. *Proteomics* 10, 3097-3107.

Ding L, Munch J, Goerls H, Maier A, Fiebig HH, Lin WH, **Hertweck C** (2010) Xiamycin, a pentacyclic indolosesquiterpene with selective anti-HIV activity from a bacterial mangrove endophyte. *Bioorg Med Chem Lett* 20, 6685-6687.

Fries A, **Winkler R**, **Hertweck C** (2010) Structural and biochemical basis for the firm chemo- and regioselectivity of the nitro-forming N-oxygenase AurF. *Chem Commun* 46, 7760-7762.

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Ishida K, **Lincke T**, **Behnken S**, **Hertweck C** (2010) Induced biosynthesis of cryptic polyketide metabolites in a *Burkholderia thailandensis* quorum sensing mutant. *J Am Chem Soc* 132, 13966-13968.

Kroiss J, Kaltenpoth M, Schneider B, **Schwinger MG**, **Hertweck C**, Maddula RK, Strohm E, Svatos A (2010) Symbiotic streptomycetes provide antibiotic combination prophylaxis for wasp offspring. *Nat Chem Biol* 6, 261-263.

Krügel H, Licht A, Biedermann G, Petzold A, Lassak J, Hupfer Y, Schlott B, **Hertweck C**, Platzer M, Brantl S, Saluz HP(2010) Cervimycin C resistance in *Bacillus subtilis* is due to a promoter up-mutation and increased mRNA stability of the constitutive ABC-transporter gene bmrA. *FEMS Microbiol Lett* 313, 155-163.

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Leone MR*, **Lackner G***, Silipo A, Lanzetta R, Molinaro A, **Hertweck C** (2010) An unusual galactofuranose lipopolysaccharide that ensures the intracellular survival of toxin-producing bacteria in their fungal host. *Angew Chem Int Ed* 49, 7476-7480. * equal contribution

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cin c resistance in *Bacillus subtilis* is due to a promoter up-mutation and increased mRNA stability of the constitutive ABC-transporter gene bmrA. *FEMS Microbiol Lett* 313, 155-163.

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Mrotzek G, Haryanti, Koesharyani I, Tretyakov AN, Sugama K, **Saluz HP** (2010) Fast short-fragment PCR for rapid and sensitive detection of shrimp viruses. *J Virol Methods* 168, 262-266.

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Department Infection Biology

Abdou R, Scherlach K, **Dahse HM**, Sattler I, Hertweck C (2010) Botryorhodines A-D, antifungal and cytotoxic depsidones from *Botryosphaeria rhodina*, an endophyte of the medicinal plant *Bidens pilosa*. *Phytochemistry* 71, 110-116.

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Memberships in Editorial Boards 2010/2011

Mitgliedschaften in Editorial Boards 2010/2011

Axel A Brakhage
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Current Genetics
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Journal of Basic Microbiology

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Journal of Genetic Syndromes and Gene Therapy

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Frontiers in Evolutionary and Population Genetics
Journal of Basic Microbiology
MycoKeys

Peter F Zipfel
Molecular Immunology
Frontiers in Molecular Innate Immunity

Lectures at the HKI 2010
Kolloquien am HKI 2010

Hans Jürgen Wester
Klinikum rechts der Isar,
TU München
Development of radioactive imaging agents exemplified on selected target/tracer concepts: transport tracers, receptor ligands and plaque imaging agents.
09.02.2010
Host: Saluz HP

Britta Qualmann
Friedrich-Schiller-Universität Jena
Shaping membranes – roles for cytoskeletal dynamics in cell morphology control
02.03.2010
Host: Brakhage AA, Stelzner A, Zipfel PF

Hans-Hartmut Peter
Medizinische Klinik der Universität Freiburg/Br
Genetik, Klinik und Diagnostik von Immundefekten beim Erwachsenen
04.05.2010
Host: Brakhage AA, Stelzner A, Zipfel PF

Jean Pierre Jacquot
Roles of redoxins in plants : from redox reactions to iron sulphur assembly
08.06.2010
Host: Saluz HP

Albert Haas
Universität Bonn
When phagocytes suffer from digestive disorder: *Rhodococcus*-containing phagosomes in macrophages
18.05.2010
Host: Brakhage AA

Christian Menge
Friedrich-Loeffler-Institut – Bundesforschungsinstitut für Tiergesundheit
Analyse zellulärer Immunreaktionen bei *E. coli*- und Mykobakterien-Infektionen des Rindes
08.06.2010
Host: Saluz HP

Nir Osherov
Tel Aviv University, Israel
There must be 50 ways to kill your fungus - antifungal discovery and novel therapeutics
10.08.2010
Host: Kniemeyer O

Sabina Heim
From an idea to a patent technology transfer at the HKI
21.09.2010
Host: Ramm M

Barbara Bröker, Uwe Völker
Universität Greifswald
Biology of *Staphylococcus aureus* in health and disease – From *in vivo* proteomics to immune proteomics
04.10.2010
Host: Zipfel PF

Iver Petersen
Friedrich-Schiller-Universität Jena
Biomarker in der Tumorpathologie
05.10.2010
Host: Brakhage AA, Stelzner A, Zipfel PF

Stefan Pelzer
B.R.A.I.N. AG, Zwingenberg
White Biotechnology - Facts, Trends & Challenges
02.11.2010
Host: Roth M

Christoph Tang
Imperial College London, London, UK
Avoidance of Innate Immune killing by *Neisseria meningitidis*
16.11.2010
Host: Zipfel PF

Claus Kroegel
Universitätsklinikum Jena
Chronisch obstruktive Bronchitis/ Emphysem – eine Autoimmunerkrankung?
23.11.2010
Host: Brakhage AA, Stelzner A, Zipfel PF

Kerstin Voigt
Jena Microbial Resource Collection (JMRC)
Zygomycetes in action: Zygomycoses as emergent diseases
07.12.2010
Host: Brakhage AA

Lectures at the HKI 2011
Kolloquien am HKI 2011

Declan Bradley
Queen's University Belfast, Royal Victoria Hospital
Regulators of the complement system and susceptibility to meningococcal disease
01.02.2011
Host: JSMC/ Zipfel PF

Michael Groll
Technische Universität München
Crystallographic knowledge in drug design strategies
22.02.2011
Host: Brakhage AA

Marc Thilo Figge
HKI
Applied Systems Biology: Dynamics, Function & Morphology
22.03.2011
Host: Brakhage AA

Thasso Griebel
Friedrich Schiller University Jena
Epos - Modular Phylogeny Framework
23.03.2011
Host: Boettger D

Richard Pott
Leibniz-Universität Hannover
Japan – Tektonischer Unruheherd: Erdbeben und Tsunamis
24.03.2011

Manfred Stamm
Leibniz-Institut für Polymerforschung Dresden
Polymers to control biofunctionality and structure at surfaces
12.04.2011
Host: Brakhage AA

Berit Jungnickel
Friedrich-Schiller-Universität Jena
Mechanismen und Regulation der somatischen Hypermutation
10.05.2011
Host: Brakhage AA, Stelzner A, Zipfel PF

Andreas Hochhaus
Universitätsklinikum Jena
Aktivierungsmechanismen der Tyrosinkinasen bei myeloproliferativen Erkrankungen
07.06.2011
Host: Brakhage AA, Stelzner A, Zipfel PF

Gabriele Pradel
Universität Würzburg
Multimeric protein complexes in the sexual stages of the malaria parasite *Plasmodium falciparum*
25.07.2011
Host: Zipfel PF

Thoralf Niendorf
Max-Delbrueck-Center for Molecular Medicine, Berlin
Experimental and clinical magnetic resonance using ultrahigh high field strengths: The better way of imaging in biology and medicine?
23.08.2011
Host: Saluz HP

Khusru Asadullah
Bayer Pharma AG
Collaborating with the pharmaceutical industry: aspects from the the "Big Pharma" perspective
22.09.11
Host: Ramm M, Briest W

Andreas Gebert
Friedrich-Schiller-Universität Jena
Intravitale 2-Phasenmikroskopie des Darmes: wie sich Lymphozyten durch Schleimhäute bewegen
04.10.2011
Host: Brakhage AA, Stelzner A, Zipfel PF

Roland Zell
Friedrich-Schiller-Universität Jena
Immer mehr Virussequenzen – wozu brauchen wir so viele Daten?
22.11.2011
Host: Brakhage AA, Stelzner A, Zipfel PF

Robert A. Cramer Jr
Montana State University, Bozeman
Hypoxia and the Pathogenesis of Invasive Pulmonary Aspergillosis: To Air or Not To Air?
29.11.2011
Host: Kniemeyer O

Scientific Awards 2010
Preise und Auszeichnungen 2010

Behnken, Swantje
medac-Forschungspreis
medac GmbH, Wedel
2010

Brunke, Sascha
Publikationspreis der Deutschsprachigen Mykologischen Gesellschaft (DMykG)
DMykG
2010

Bruns, Sandra
medac-Forschungspreis
medac GmbH, Wedel
2010

Ishida, Keishi
medac-Forschungspreis
medac GmbH, Wedel
2010

Jacobsen, Ilse D.
Posterpreis der Deutschsprachigen Mykologischen Gesellschaft (DMykG)
DMykG
2010

Józsi, Mihály
Publikationspreis des XLVII ERA-EDTA Congress / II DGfN Congress München
2010

Kniemeyer, Olaf
medac-Forschungspreis
medac GmbH, Wedel
2010

Kusebauch, Björn
Posterpreis des International VAAM Workshop „Biology of bacteria producing national products“, Tübingen
VAAM
2010

Lackner, Gerald
medac-Forschungspreis
medac GmbH, Wedel
2010

Lincke, Thorger
medac-Forschungspreis
medac GmbH, Wedel
2010

Lincke, Thorger
Posterpreis der 22. Irseer Naturstoff-Tage
DECHEMA
2010

Möllmann, Ute
Thüringer Forschungspreis
Thüringer Ministerium für Bildung Wissenschaft und Kultur
2010

Opfermann, Thomas
Siemens Award Excellence in Preclinical Imaging at World Molecular Imaging Congress 2010
Siemens AG
2010

Roth, Martin
medac-Forschungspreis
medac GmbH, Wedel
2010

Scharf, Daniel
Posterpreis der 10th European Conference on Fungal Genetics, Leiden, Netherlands
DSM
2010

Scharf, Daniel
Posterpreis des EMBO Practical Course “Protein Expression, Purification and Crystallisation” PEPC7, Hamburg
2010

Staib, Peter
Publikationspreis der Deutschsprachigen Mykologischen Gesellschaft (DMykG)
DMykG
2010

Strobel, Stefanie
Posterpreis der 2nd International Conference HUS-MPGN-PNH, Innsbruck, Austria
Novartis Pharma GmbH
2010

Thywißen, Andreas
medac-Forschungspreis
medac GmbH, Wedel
2010

Scientific Awards 2011
Preise und Auszeichnungen 2011

Behnken, Swantje
Oral presentation award of the ILRS Symposium, Dornburg
ILRS
2011

Brock, Matthias
Pettenkofer-Preis
Pettenkofer-Stiftung
2011

Brunke, Sascha
Hans-Rieth-Posterpreis der Deutschsprachigen Mykologischen Gesellschaft (DMykG)
DMykG
2011

Chen, Qian
medac-Forschungspreis
medac GmbH, Wedel
2011

Enghardt, Tina K.
Oral presentation award of the ILRS Symposium, Dornburg
ILRS
2011

Föge, Martin
Posterpreis der Deutschsprachigen Mykologischen Gesellschaft (DMykG)
DMykG
2011

Föge, Martin
Poster award of the International Conference on Microbial Communication (MICOM), Jena
JSMC
2011

Hartmann, Andrea
medac-Forschungspreis
medac GmbH, Wedel
2011

Hertweck, Christian
NPR Lecture Award, ASP Meeting San Diego, USA
Natural Product Reports
2011

Horn, Fabian
medac-Forschungspreis
medac GmbH, Wedel
2011

Grumbt, Maria
Wissenschaftspreis 2011 für eine herausragende Publikation auf dem Gebiet der medizinischen Mykologie
DMykG
2011

Kroll, Kristin
Talk award International Conference on Microbial Communication (MICOM), Jena
JSMC
2011

Lopez, Crisanto M.
Poster award of the ILRS Symposium, Dornburg
ILRS
2011

Luo, Shanshan
Wissenschaftspreis 2011 für eine herausragende Publikation auf dem Gebiet der medizinischen Mykologie
DMykG
2011

Nützmann, Hans-Wilhelm
medac-Forschungspreis
medac GmbH, Wedel

Rudolph, Martin
Posterpreis des International VAAM Workshop “Biology of bacteria producing national products“, Karlsruhe
VAAM
2011

Scharf, Daniel
Oral presentation award ESF-EMBP Symposium on “Synthetic Biology of Antibiotic Production”, Sant Feliu Guixols, Spain
ESF-EMBP Symposium
2011

Scherlach, Kirstin
medac-Forschungspreis
medac GmbH, Wedel
2011

Schroeckh, Volker
medac-Forschungspreis
medac GmbH, Wedel
2011

Schümann, Julia
medac-Forschungspreis
medac GmbH, Wedel
2011

Schwartze, Volker U und Jacobsen, Ilse D.
Outstanding Poster Presentation Award der Korean Mycological Society
Korean Mycological Society
2011

Thön, Marcel
Wissenschaftspreis für Lebenswissenschaften und Physik des Beutenberg-Campus Jena e.V.
Beutenberg-Campus Jena e.V.
2011

Voigt, Kerstin
Outstanding Poster Presentation Award der Korean Mycological Society
Korean Mycological Society
2011

Meetings, Workshops, Symposia 2010/2011
Wissenschaftliche Veranstaltungen 2010/2011

Bioorganic Chemistry Symposium
Hertweck C
Jena, Germany
2010

Irseer Naturstofftage
Hertweck C *et al.*
Irsee, Germany
2010

IFO-SFI - Interdisciplinary Forum on Superficial Fungal Infections
Zipfel PF, Hube B, Kurzai O, Staib P, Hipler U-C
Jena, Germany
2010

ILRS Group Seminar
Schmidt D, Zipfel PF
Jena, Germany
2010

European Student Conference on Microbial Communication
Nützmann HW, Thywissen A, Heddergott C, Linde J, Otzen C *et al.*
Jena, Germany
2010

25 Jahre VAAM Festsymposium
Brakhage AA
Jena, Germany
2010

Systems Biology for Industry: Dynamics and regulation of the metabolic balance in *Escherichia coli*
Guthke R *et al.*
2010

Irseer Naturstofftage
Hertweck C *et al.*
Irsee, Germany
2011

3rd Postgraduate Symposium on Cancer Research
Zipfel PF *et al.*
Jena, Germany
2011

ILRS Symposium
Schmid D, Zipfel PF
Dornburg, Germany
2011

ILRS Group Seminar
Vogler C, Zipfel PF
Jena, Germany
2011

FINSysB Research Skills Training Workshop State-of-the-art infection models
Hube B, Miramón P, Wilson D, Jacobsen ID, Wächtler B, Schild L, Lüttich A, Stöckel U, Weber B
Jena, Germany
2011

Minisymposium Antibiotic resistome in the food chain
Krügel H, Saluz HP *et al.*
Jena, Germany
2011

International Conference on Microbial Communication
Figge, MT
Jena, Germany
2011

International Workshop on Systems Biology of Microbial Infection
Guthke R, Figge MT
Jena, Germany
2011

Invasive Mycoses in Hematological Malignancies - IMIHM V
Kurzai O *et al.*
Würzburg, Germany
2011

Experimental Mycology/Fungal Biology and Biotechnology VAAM Fachgruppenseminar
Brock M et. al.
Karlsruhe, Germany
2011

Graduations 2010
Promotionen 2010

Albrecht, Daniela
Integration of transcriptome and proteome data from human-pathogenic fungi
Friedrich-Schiller-Universität Jena

Anssour, Samir
Functional consequences of natural and synthetic polyploidization of *Nicotiana attenuata* and *N. obtusifolia* - special focus on expressed anti-herbivore response
Friedrich-Schiller-Universität Jena

Behnsen, Judith
Interaction of *Aspergillus fumigatus* with the immune system with special focus on the complement system
Friedrich-Schiller-Universität Jena

Bergmann, Sebastian
Regulation of secondary metabolite gene clusters in *Aspergillus nidulans*
Friedrich-Schiller-Universität Jena

Brunke, Sascha
Molekularbiologische Untersuchungen zur Pigmentsynthese von humanpathogenen Hefen
Freie Universität Berlin

Busch, Benjamin
Functional analyses and engineering of the aureothin and rhizoxin biosynthetic pathways
Friedrich-Schiller-Universität

Fleck, Christian
Untersuchungen zur Redundanz im Pilzmetabolismus am Beispiel von *Aspergillus nidulans*, *Aspergillus fumigatus* und *Saccharomyces cerevisiae*
Friedrich-Schiller-Universität Jena

Gropp, Katharina
Auseinandersetzung zwischen der humanpathogenen Hefe *Candida albicans* und dem angeborenen Immunsystem des Wirts
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Hecker, Michael
Integrative modeling of transcriptional regulation in response to autoimmune disease therapies
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Ludwig, Mirko
Funktionelle Charakterisierung des Faktor H verwandten Proteins CFHR3
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Luo, Shanshan
Candida albicans and host interaction: the many faces of the Candida pra1 protein
Friedrich-Schiller-Universität Jena

Onkokesung, Nawaporn
Jasmonic acid and ethylene crosstalk: Regulation of growth, defense and metabolisms in native tobacco *Nicotiana attenuata* in response to herbivory
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Richter, Martin E.A.
Biochemische Charakterisierung multifunktioneller Oxygenasen aus mikrobiellen Sekundärmetabolit-Biosynthesewegen
Friedrich-Schiller-Universität

Schmidt, André
Die Stressantwort von *Aspergillus fumigatus* auf Eisenmangel: Vergleichende Proteomanalysen und die Charakterisierung Eisen-regulierter Proteine
Friedrich-Schiller-Universität Jena

Strobel, Stefanie
The role of complement autoantibodies in hemolytic uremic syndrome and dense deposit disease
Friedrich-Schiller-Universität Jena

Thön, Marcel
Redox regulation of the *Aspergillus nidulans* CCAAT-binding factor (AnCF)
Friedrich-Schiller-Universität Jena

Unger, Michelle
Molekularbiologische Grundlage der Cervimycin-Biosynthese in *Streptomyces tendae*
Friedrich-Schiller-Universität

Graduations 2011
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Abdou, Randa
Bioactive secondary metabolites from the endophytic microorganisms of the medicinal plant *Bidens pilosa*
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Sekundärmetabolite von Actinomyceten aus belasteten Innenräumen
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Borth, Nicole
Funktionelle Charakterisierung neuer Interaktionen zwischen chlamydialen Proteinen und Wirtszellproteinen
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Isolation and characterization of mine-dwelling actinomycetes as potential producers of novel bioactive secondary metabolites
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The role of the interactions of human complement factor H-related protein 4 with C-reactive protein and C3b in the modulation of complement activation
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Jain, Radhika
The Map Kinase A (MpkA) regulated cell wall integrity signaling pathway in the pathogenic fungus *Aspergillus fumigatus*
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Das Genom des endofungalen Bakteriums *Burkholderia rhizoxinica* – molekulare Grundlagen der Bakterien-Pilz-Interaktion
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Lambeck, Sandro
Systembiologische Charakterisierung der organübergreifenden Transkriptomantwort in muriner Sepsis und die Modellierung bimodaler Muster
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Lauer, Nadine
Die Rolle des Komplementsystems bei Entstehung der altersabhängigen Makuladegeneration.
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Lincke, Thorger Leif
Isolierung und Strukturaufklärung kryptischer Sekundärmetabolite aus *Burkholderia thailandensis* und *Clostridium cellulolyticum*
Friedrich-Schiller-Universität Jena

Losse, Josephine
The interaction of neutrophil granulocytes with *Candida albicans*: Role of complement receptor 3 ligands in modulating host cell responses
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Reuter Michael
Die Regulation des Komplementsystems im Rahmen von Infektionen.
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Sarkar, Anindita
Expression of silent secondary metabolite gene cluster in the filamentous fungi *Aspergillus nidulans*
Friedrich-Schiller-Universität Jena

Schild, Lydia
Einfluss zelloberflächenassoziierter Proteasen auf die Zellbiologie und Pathogenität von *Candida albicans*
Friedrich-Schiller-Universität Jena

Schindler, Susann
Immune evasion strategies of dermatophytes
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Schöbel, Felicitas
Lysinbiosynthese von *Aspergillus fumigatus*: Virulenzstudien und biochemische Analysen
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Tchize Ndejouong, Basile Le Sage
Isolation, structure elucidation and biological evaluation of metabolites from *Streptomyces* spp. dwelling in extreme and unusual habitats
Friedrich-Schiller-Universität Jena

Vödisch, Martin
Anpassung von *Aspergillus fumigatus* an hypoxische Bedingungen: Vergleichende Proteomanalysen und Charakterisierung Hypoxie-regulierter Proteine
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Volling, Katrin
The human pathogenic fungus *Aspergillus fumigatus* inhibits apoptosis in alveolar macrophages
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Wächtler, Betty
Molekulare Charakterisierung der Invasionsmechanismen von *Candida albicans* bei oberflächlichen Infektionen
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Wollbold, Johannes
Attribute exploration of gene regulatory processes
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Bachelor / Master / Diploma Theses 2010
Bachelor-/ Master-/ Diplomarbeiten 2010

Altwasser, Robert
Large-scale network inference for *Candida albicans* using LASSO-based methods
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Arlt, Janine
Gibt es einen Zusammenhang zwischen Wirtsmikrobiom und Monodon slow growth syndrome (MSGS)
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Untersuchung zur Rolle des *EED1*-Gens während des filamentösen Wachstums von *Candida albicans*
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SNP analysis of rheumatoid arthritis patients with different responsiveness to the anti-TNF-therapy
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Haedicke, Katja
Interaktion von *Candida albicans* mit oralen Epithelzellen: Charakterisierung infektionsassoziierter Gene
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Heurich, Adeline
TypIII-sekretorische Proteine von zoonotischen Chlamydien und ihre Interaktion mit Wirtsproteinen
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Hotze, Melaniel
In silico Sequenzoptimierung synthetischer Gene und Zuordnung des Expressionslevels über Transkriptionsanalysen am Beispiel von *Candida albicans*
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Micklisch, Sven
Funktionelle Charakterisierung von krankheits-assoziierten Proteinen bei der Altersabhängigen Makuladegeneration
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Mock, Marlen
Einfluss von Aspartatproteasen auf zelluläre Funktionen von *Candida albicans*
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Müller, Christiane
Molekulare Untersuchungen zur genetischen Variabilität von *Peneaus monodon*
Friedrich-Schiller-Universität Jena

Pähtz, Vera
Identifizierung und Charakterisierung von Sporen-assoziierten Proteinen des humanpathogenen Pilzes *Aspergillus fumigatus*
Friedrich-Schiller-Universität Jena

Rudolph, Martin Michael
Molecular engineering and evaluation of reporter strains for high-throughput drug screening on a microfluidic platform
Friedrich-Schiller-Universität Jena

Schisnowski, Daniel
Etablierung einer Methode zur gerichteten Evolution mittels Phage display
Fachhochschule Jena

Simon, Svenja
Multivariate Analyse von durchflusszytometrischen Daten
Friedrich-Schiller-Universität Jena

Sugimoto, Yuki
Untersuchung der Biosynthese von Aureothin und Neoaureothin
Friedrich-Schiller-Universität Jena

Vlaic, Sebastian
Analysis of proteome data of stimulated fibroblasts from rheumatoid arthritis and osteoarthritis patients
Friedrich-Schiller-Universität Jena

Weber, Michael
Network inference from transcriptome monitoring of the response of synovial fibroblasts from rheumatoid arthritis and osteoarthritis Patients to TNFalpha
Friedrich-Schiller-Universität Jena

Wolf, Bibiane
Studien zur chemischen Derivatisierung von Rhizoxin
Freie Universität Berlin

Bachelor / Master / Diploma Theses 2011
Bachelor-/ Master-/ Diplomarbeiten 2011

Bergmann, Holger
Sequenzbasierte Untersuchungen fungaler PKS-NRPS-Biosynthe-seenzmye
Friedrich-Schiller-Universität Jena

Bettenhäuser, Sandra
Molekulare Untersuchungen zur Funktion der Transkriptionsregulatoren StuA, SteA und MedA des humanpathogenen Dermatophyten *Arthroderma benhamiae*
Friedrich-Schiller-Universität Jena

Boldt, Sabrina
Design von Siderophor-basierten und Calcofluor-basierten Polyen-Antimykotika-Konjugaten
Friedrich-Schiller-Universität Jena

Franke, Jakob
Isolierung und Strukturaufklärung von Sekundärmetaboliten aus *Burkholderia thailandensis*
Ludwig-Maximilians-Universität München

Geib, Elena
Biosynthese von Terrein in *Aspergillus terreus*
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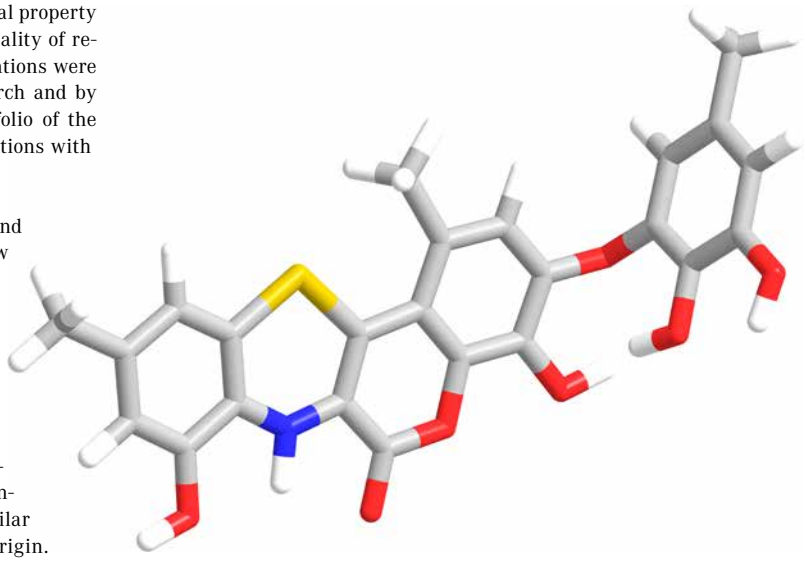
Friedrich-Schiller-Universität Jena

Inventions and Patents 2010/2011
Erfindungen und Schutzrechte 2010/2011

In addition to publications in peer reviewed journals, intellectual property rights are the main performance parameters reflecting the quality of research at the HKI. In 2010/2011 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.

The Departments Biomolecular Chemistry and Molecular and Applied Microbiology explored in a close collaboration a new approach to trigger metabolite production by deleting a gene encoding the N-acetyltransferase NnaB in *Aspergillus nidulans*. A metabolic switch was thus induced, which allowed the isolation and structural elucidation of unparalleled fungal metabolites, named pheofungins A-D (see Figure). Both teams provided a body of evidence that these red pigments are the fungal response to global stress caused by impaired posttranslational modification. The structures of the pheofungins represent benzopyranobenzothiazinones that are fully unprecedented for fungi. However, the pheofungin chromophore is remarkably similar to pheomelanins, the red pigments in human hair of Celtic origin. Transcription analysis and gene knock-out experiments indicated that pheofungins result from the condensation of orsellinic acid derived phenols with cysteine in analogy to the pathway for red hair pigments. From a pharmacological point of view, the discovery of the novel benzopyranobenzothiazinones is significant because of the potent antiproliferative activity of pheofungin C. This study thus not only describes a new avenue to structurally intriguing metabolites but also illustrates that modulation of fungal regulatory systems can promote the discovery of natural products with potential therapeutic application.



Targeted modification of the regulation of fungal secondary metabolism in *Aspergillus nidulans* enabled the discovery of the pharmaceutically interesting pheofungins.

The application for new patents is stringently evaluated within the HKI and focuses on novel biologically active natural products and their (bio-) synthetic derivatives. The HKI closely co-operates with Ascenion GmbH, Munich, to further optimize technology transfer and commercialization activities.

Heinen S, Lambris JD, Lauer N, **Skerka C**, **Zipfel PF** (2010)
Potent inhibitors of complement activation
US 61/432,240

Brakhage AA, **Dahse HM**, **Hertweck C**, **Nützmann H-W**, **Scherlach K**, **Schroeckh V** (2011)
Pheofungins
EP 11 005 242.0

Kusebauch B, **Dahse HM**, **Hertweck C**, **Kirchner H**, **Scherlach K**, Fiebig HH, Kelter G, Maier A (2011)
Neue Rhizoxinderivate
DE 10 2012 000 956.9

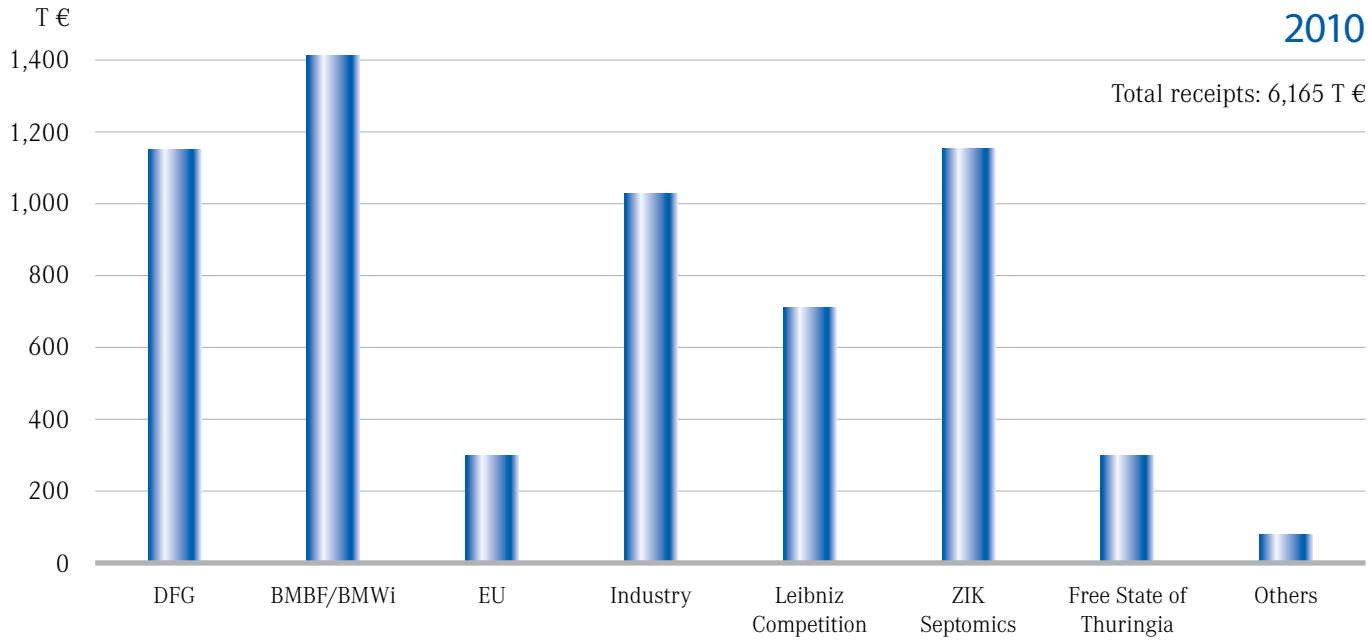
Ueberschaar N, **Tchize Ndejouong BLS**, **Ding L**, **Hertweck C**, Fiebig HH, Kelter G, Maier A (2011)
Hydrazidomycins
EP 11 004 267.8

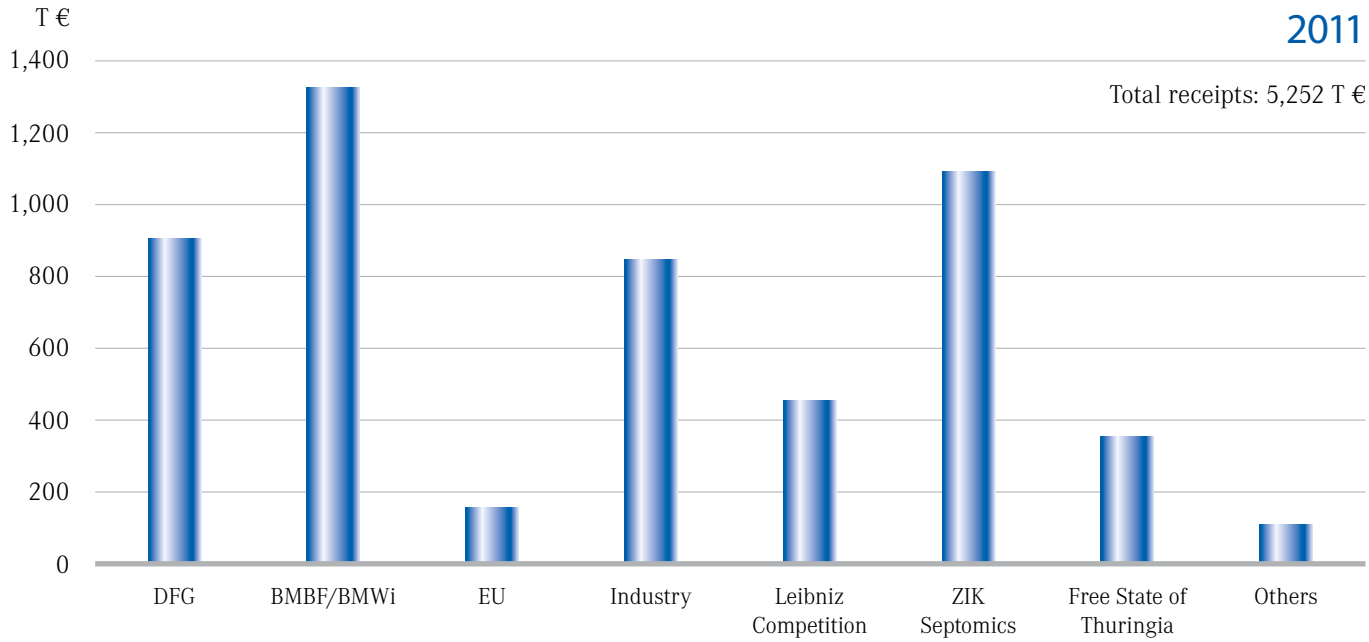
Schutzrechte sind neben Originalpublikationen in referierten Fachjournalen ein wesentlicher Leistungsparameter für die Forschungsarbeit am HKI. Sowohl die naturstoffchemisch arbeitenden Abteilungen als auch Technologie-orientierte Gruppen trugen im Zeitraum 2010/2011 mit einer Reihe von Erfindungen zum 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.

Teams aus den Abteilungen Biomolekulare Chemie und Molekulare und Angewandte Mikrobiologie wählten einen neuen Ansatz zur Aktivierung der Wirkstoffproduktion in *Aspergillus nidulans*, indem sie das für N-Acetyltransferase NnaB codierende Gen ausschalteten. Die damit einhergehende Umsteuerung des Metabolismus ermöglichte die Isolierung bislang unbekannter pilzlicher Stoffwechselprodukte die als Pheofungine A-D bezeichnet wurden (s. Abb.). Die Wissenschaftler um Axel Brakhage und Christian Hertweck konnten zeigen, dass der Pilz diese rot gefärbten Substanzen als Antwort auf Stresssituationen bildet, die durch eine Beeinträchtigung posttranslationaler Modifikationen ausgelöst werden. Pheofungine gehören zur Stoffgruppe der bei Pilzen bisher gänzlich unbekannten Benzpyranobenzothiazinone. Sie gleichen den Pheomelaninen, die für die rote Haarfarbe von Menschen keltischer Abstammung verantwortlich sind. Molekularbiologische Untersuchungen zeigten, dass Pheofungine analog der Synthese des roten Haarpigments aus der bekannten Orsellinsäure hervorgehen. Aus pharmakologischer Sicht ist die Entdeckung der neuen Benzpyranobenzothiazinone vor allem deshalb interessant, weil Pheofungin C über eine starke antiproliferative Aktivität verfügt. Die Entdeckung der Pheofungine weist einerseits einen neuen methodischen Zugang zu den in der Natur vorhandenen Naturstoff-Ressourcen und zeigt zudem, wie durch gezielte Beeinflussung der pilzlichen Stoffwechselregulation neue, möglicherweise therapeutisch nutzbare Wirkstoffe gefunden werden können.

Die Anmeldung neuer Schutzrechte unterliegt einer strengen hausinternen Evaluation und konzentriert sich auf neue, biologisch aktive Naturstoffe und deren (bio-)synthetische Derivate. Das HKI arbeitet zur effektiven Verwertung der Schutzrechte erfolgreich mit der Ascenion GmbH, München zusammen.

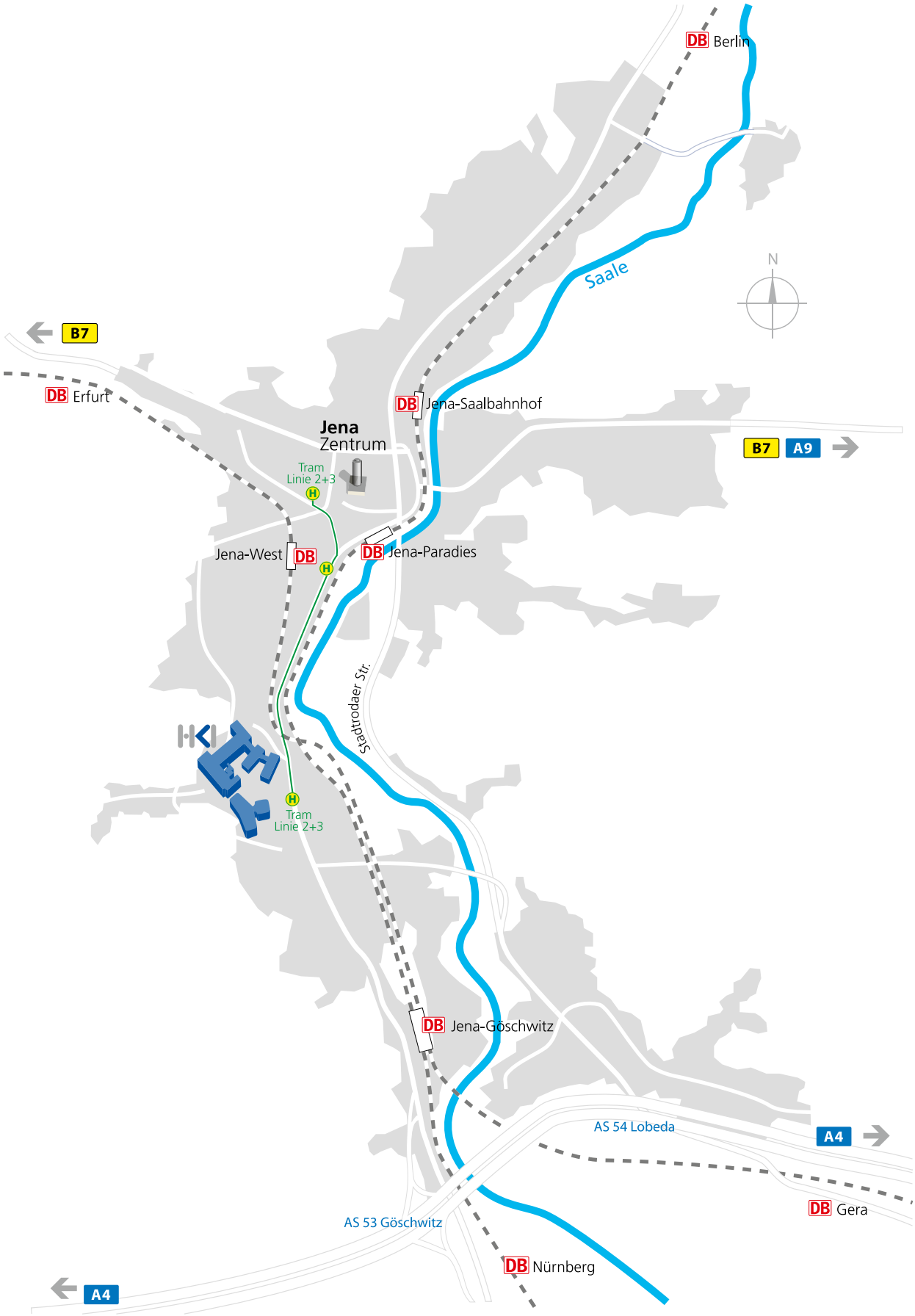
External Funding 2010/2011
Drittmittel 2010/2011

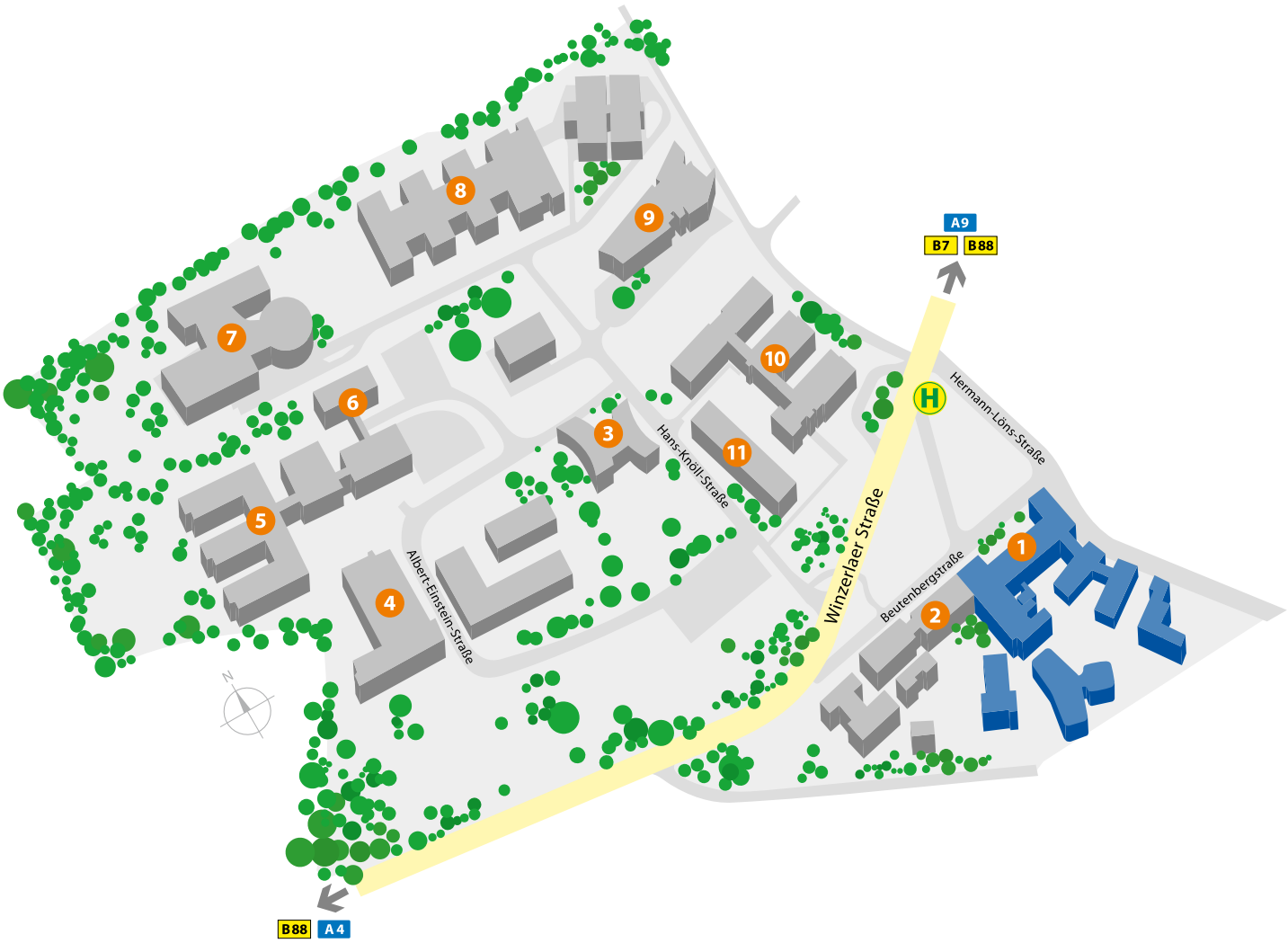




Third-party funds during the period reported: composition of the sources of income
Drittmittelausgaben im Berichtszeitraum: Verteilung über die Drittmittelgeber

Maps | Lagepläne





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