

# 2nd ILRS Symposium

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**September 15-16, 2008**

Location: Altes Schloss, Dornburg

## Monday September 15, 2008

- 12:00** Departure by bus from Jena to Dornburg Castle  
(meeting point & departure: backside of the bus station "Beutenberg Campus")  
**12:10** bus stop city centre "Bibliotheksweg" backside of the ThULB)

Snack bar

13:00 Welcome and Announcements  
Peter Zipfel, spokesperson of the ILRS

### 13:30 Plenary Lecture

Ingo Autenrieth, Universitätsklinikum Tübingen, Inst. für Medizinische Mikrobiologie und Hygiene

Immune evasion mechanisms of *Yersinia enterocolitica*.

### 1st Session, Networks, Interactions and their analysis I

Chair: Susann Erdmann

- |       |                   |   |
|-------|-------------------|---|
| 14:20 | Radhika Jain      | Regulation of cell wall integrity signalling by mitogen-activated protein kinase MpkA in <i>Aspergillus fumigatus</i> |
| 14:40 | YongQiang Wang    | Interaction of floral homeotic proteins: an evolutionary view   |
| 15:00 | Daniela Albrecht  | Missing data in gel-based proteomics  |
| 15:20 | Christian Hummert | Improving the Quality of Microarray Analysis  |
| 15:40 | Felicitas Schöbel | Nutrition during pathogenesis of <i>Aspergillus fumigatus</i> : Impact of <i>de novo</i> synthesis of lysine          |

16:00 Coffee Break

## 2nd Session, Networks II and Interactions of pathogenic microorganisms with hosts I

Chair: Daniela Albrecht

- 16:30 Stefanie Seitz Temperature Integration of the Circadian RNA-Binding Protein CHLAMY1
- 16:50 Krisztina Truta-Feles Characterization of novel chemotactic factors for  $\gamma\delta$  T-lymphocytes
- 17:10 Shanshan Luo Characterization of CaCRASP-2, a novel surface protein of *Candida albicans* which mediates immune evasion and adhesion to host cells
- 17:30 Hoang Hoa Long Diversity of natural endophytic bacteria in *Nicotiana attenuata* roots depends on plant genotype and soil type
- 17:50 Jennifer Sneed Growth inhibition of ecologically relevant bacterial species by the green alga, *Dictyosphaeria ocellata*.
- 18:15 Poster Session**
- 20:00 *Get together*
- 22:30 *Return to Jena by bus*

## Tuesday September 16, 2008

**8:15** *Departure by bus from Jena to Dornburg Castle (meeting point & departure: backside of the bus station "Beutenberg Campus" 8:25 bus stop city centre "Bibliotheksweg" backside of the ThULB)*

### 9:00 Plenary Lecture

Holger Deising, Martin-Luther-Universität Halle-Wittenberg, Inst. für Agrar- und Ernährungswissenschaften, Phytopathologie und Pflanzenschutz  
Molecular dissection of the *Colletotrichum* infection

## 3rd Session, Interactions of pathogenic microorganisms with hosts II

Chair: Hoang Hoa Long

- 9:50 François Mayer Identification and characterisation of infection-associated genes in *Candida albicans*
- 10:10 Hangxing Yu Role of PARP-1 cleavage in *Chlamydia trachomatis*-induced host cell death
- 10:30 Katrin Volling Apoptosis inhibition of alveolar macrophages upon interaction with conidia of *Aspergillus fumigatus*.
- 10:50 *Coffee Break*

#### 4th Session, Interactions between microorganisms I

Chair: Jennifer Sneed

- 11:20 Christoph Heddergott Analysis of proteins secreted by the Dermatophyte fungus *Arthroderma benhamiae*
- 11:40 Anindita Sarkar Real Time Monitoring of Secondary Metabolite Gene Expression in *Aspergillus nidulans*
- 12:00 Lidan Ye Lignin degradation: From aerobic fungal halogenation to anaerobic bacterial dehalogenation
- 12:20 Gerald Lackner Genomic studies of the endofungal, rhizoxin producing bacterium *Burkholderia rhizoxinica*.
- 12:40 *Lunch*

#### 5th Session, Interactions between microorganisms II

Chair: Stefanie Seitz

- 13:30 Susann Erdmann Real-time PCR for detection of pheromone and receptor gene expression in the basidiomycete *Schizophyllum commune*
- 13:50 Alexander Funk Regulation of Polyketide Synthase Gene Clusters in *Aspergillus nidulans*
- 14:10 Anne Behrend Heavy metal ion stress – Induction of chemical differentiation in *Streptomyces coelicolor*
- 14:45 Final Remarks
- 15:00 *Return to Jena by bus*

Plenary Lecture: **Immune evasion mechanisms of *Yersinia enterocolitica*.**

**[Ingo Autenrieth](#)**

Universitätsklinikum Tübingen, Inst. für Medizinische Mikrobiologie und Hygiene

## Regulation of cell wall integrity signalling by mitogen-activated protein kinase MpkA in *Aspergillus fumigatus*

[Radhika Jain](#), Axel A. Brakhage, Dept. Molecular and Applied Microbiology, HKI

Mitogen-activated protein kinase (MAPK) cascades are evolutionary conserved signalling modules transducing stimuli from the cell surface to the nucleus. MAPK pathways control key virulence functions, including host-induced spore germination, polarised hyphal growth, adhesion to the host surface, differentiation of specialised infection structures, remodelling of the fungal cell wall or secretion of enzymes and toxins. The genome of *A. fumigatus* harbours four MAPK genes: *sakA/hogA*, *mpkA*, *mpkB* and *mpkC*. SakA has been shown to be involved in conidial germination under nitrogen and carbon source starvation. MpkC, sharing similar functions to SakA, is involved in sensing alternative carbon sources. We are investigating the role of mitogen activated protein kinase MpkA in *Aspergillus fumigatus*. For this purpose, deletion of *mpkA* was carried out in *A. fumigatus* and the mutant strain was phenotypically analysed. To study the transcriptional regulation of *mpkA*, an *A. fumigatus* strain was generated carrying the *lacZ*-reporter gene fused to the *mpkA*-promoter. Transcriptional read out was done using a beta-galactosidase assay. Post transcriptional modification of MpkA was monitored by western blot analysis. Here, we present data on the transcriptional regulation of *mpkA*, and the function of the corresponding protein in *A. fumigatus*. □ *mpkA* mutants are defective in cell wall formation, sensitive to menadione and diamide but more tolerant to H<sub>2</sub>O<sub>2</sub>. Furthermore, induction of *mpkAp-lacZ* was observed by diverse cell-wall perturbing agents, e.g., glucanex and SDS. In contrast, western blot analysis showed post-transcriptional regulation for MpkA in response to some reactive oxygen species. In conclusion, MpkA plays an important role in the cell-wall integrity signalling pathway of *A. fumigatus* and also shows transcriptional and post-transcriptional regulation under different stress conditions.

## Interaction of floral homeotic proteins: an evolutionary view

[YongQiang Wang](#), Günter Theißen, Dept. of Genetics, FSU

The identity of floral organs in angiosperms is specified by multimeric transcription factor complexes composed of MADS-domain proteins that bind to specific *cis*-regulatory elements (“CArG-boxes”) of their target genes, thus constituting “floral quartets”. Gymnosperms, angiosperms’ closest relatives, contain orthologues of floral homeotic genes, but when and how the interactions constituting floral quartets were established during evolution has remained unknown. To better understand the “abominable mystery” of flower origin we have comprehensively studied the dimerization and DNA-binding of several classes of MADS-domain proteins from a gymnosperm, the gnetophyte *Gnetum gnemon*. Determination of protein-protein interactions by both yeast two-hybrid (which has been done previously by others in our lab) and *in vitro* pull-down assays revealed complex patterns of heterodimerization among orthologues of class B (DEFICIENS/GLOBOSA-like), class C (AGAMOUS-like), Bsister (ABS/TT16-like) floral homeotic proteins and AGL6-like proteins, while homodimerization was observed only in exceptional cases. In contrast, electrophoretic mobility shift assays (EMSAs) revealed that all proteins tested except one were found to bind to CArG-boxes also as homodimers, suggesting that homodimerization is relatively weak, but facilitated by DNA-binding. DNA-based homodimerization includes orthologues of class B (B) and class C (C) proteins; B and C proteins form also heterodimers *in vitro* and in yeast, which is in sharp contrast to their orthologues from angiosperms, which require class E (SEPALLATA-like) proteins to “glue” them together in multimeric complexes. Remarkably, the heterodimers of B and C proteins from *Gnetum* are not capable of binding to CArG-boxes, suggesting that DNA-binding *in vivo* is based on homodimers, while heterodimerization of B and C proteins may constitute multimeric, DNA-bound complexes. Our data suggest that gymnosperm B and C proteins control male organ identity by forming a quartet complex composed of two homodimers, each bound to a CArG-box. Since class E proteins are very likely absent from gymnosperms, our findings suggest that the origin of higher order complex formation of floral homeotic proteins is based on the interaction of different DNA-bound homodimers, and predates the origin of angiosperms and the establishment of class E genes. Further experiments (BiFC and *in vitro* footprinting) to test these hypothesis are under going.

## Missing data in gel-based proteomics

[Daniela Albrecht](#), Reinhard Guthke, Dr. Ulrich Möller, Dept. Molecular and Applied Microbiology, Systems Biology / Bioinformatics, HKI

Gel-based proteomics is a widely applied technique to measure abundances of proteins in various biological systems. Comparison of two or more biological groups involves matching of 2-D gels. Depending on the software, this can result in spots showing missing values on several gels. Most studies ignore this fact or substitute all missing data by zero. Since a couple of years, scientists have realized that this is not the optimal way of analyzing their data and several studies were published presenting methods of imputing missing proteomics data. Most of these methods have already been applied to microarray data before; the phenomenon of missing data is well known in this field, too.

In this talk, I want to further raise awareness of the problem of missing values in gel-based proteomics. Reasons for missing values are summarized and their distribution in datasets is explored. Also, a comparison and evaluation of hitherto proposed imputation methods for gel-based proteomics data is provided.

## Improving the Quality of Microarray Analysis

[Christian Hummert](#), Reinhard Guthke, Dr. Ulrich Möller, Dept. Molecular and Applied Microbiology, Systems Biology / Bioinformatics, HKI

Department Molecular and Applied Microbiology, Leibniz Institute for Natural Product Research and Infection Biology, Hans-Knoell-Institute, Beutenbergstr. 11a, D-07745 Jena, Germany

### Motivation

Microarray experiments always contain high error rates [E06]. On the one hand there are systematic errors like cross-hybridizations or intron matching probesets. On the other hand unsystematic errors introduced by a specific experiment are more difficult to handle. To cope with this problems new chip definition files (CDFs) avoiding cross-hybridizations have been created, an machine learning approach to overcome unsystematic errors has been developed and work on new chip designs is ongoing.

### Results

New CDFs have been created for different Affymetrix chips. These CDFs result from Boolean terms, which avoid cross-hybridization completely. They have been compared to the original Affymetrix CDFs and to results from other groups like Ferrari et al. [F07]. The Affymetrix GeneChip data from a concrete experiment from Koczan et al [K08] was analysed using the different CDFs. The obtained results were correlated to qRT-PCR measurement readings for the same experiment. The Boolean probeset Reassembly CDFs have the highest correlation co-efficients. Availability: The new CDFs are freely available as R-packages in the Comprehensive R Archive Network (RCRAN) and are easy to use. The machine learning algorithms are able to generalize the error information calculated from those genes, qRT-PCR data are available for, to all others. The probesets could be assigned to different categories of reliability. As result untrustable probesets can be ignored and on the other hand the remaining probesets provide more confidence.

### References

- E06. Michael Eisenstein (2006): Technology Feature Microarrays — Quality Control. In: Nature, 442:31, 1067-1072
- F07. Ferrari, S. et al.(2007): Novel Definition Files for Human GeneChips Based on GeneAnnot. In: BMC Bioinformatics, 8(1), 446.
- K08. Koczan, S. et al. (2008): Molecular Discrimination of Responders and Non-Responders to Anti-TNF $\alpha$  Therapy in Rheumatoid Arthritis. In: Arthritis Research & Therapy, 10, R50.

## Nutrition during pathogenesis of *Aspergillus fumigatus*: Impact of *de novo* synthesis of lysine

[Felicitas Schöbel](#), Matthias Brock, Axel A. Brakhage, New research group - Microbial Biochemistry and Physiology, HKI

*A. fumigatus* is a saprophytic fungus which is able to cause severe infections in immunocompromised patients, such as invasive aspergillosis. Diagnosis of this disease is difficult and therapy is hampered by severe side effects of antifungal drugs. We are interested in the carbon and energy sources utilised by the fungus during invasive growth within the lung and analyse the specific enzymes involved in their degradation. Among other pathways we investigate the alpha-aminoadipate pathway, which leads to the formation of lysine. Since the key enzymes of this pathway are absent in humans and could, therefore, be possible targets for new antifungal drugs, we study the role of these enzymes in pathogenesis. In this study, we focused on the homocitrate synthase, catalysing the first step of the lysine biosynthesis. Growth experiments revealed that a deletion mutant was able to grow on serum, as well as on protein containing media, which were previously treated with *A. fumigatus* proteases, indicating that the overall lysine content released from host proteins should be sufficient to support growth of the mutant. However, a deletion mutant of the homocitrate synthase displayed a strongly attenuated virulence, when tested in a murine infection model, but virulence was mainly restored by feeding lysine with the drinking water. This indicates that the *de novo* synthesis of lysine is essential for the establishment of invasive aspergillosis and required for the germination of conidia within the host tissue.

## Temperature Integration of the Circadian RNA-Binding Protein CHLAMY1

[Stefanie B. Seitz](#), Olga Voytsekh, Dobromir Iliev, Maria Mittag, Inst. General Botany, FSU

The circadian RNA-binding protein CHLAMY1 from the green alga *Chlamydomonas reinhardtii* consists of two subunits that contain either three Lysine-homology- (C1 subunit) or three RNA-recognition-motifs (C3 subunit) [1]. Changes in the C1 level cause arrhythmicity of the phototaxis rhythm while alterations in the level of C3 lead to acrophase shifts. Thus, CHLAMY1 is involved in maintaining period and phase of the *C. reinhardtii* circadian clock [2]. We have analyzed if the two subunits do also play a role in temperature integration, the basis for other key properties of circadian clocks including entrainment by temperature cycles and temperature compensation. We can show that C1 is hyper-phosphorylated at low and hypo-phosphorylated at high temperature. In case of C3, its expression level is up-regulated at low temperature *via* transcriptional regulation involving predominantly an E-, but also DREB1A-boxes. These data suggest that a temperature controlled functional network of clock-relevant proteins exists in *C. reinhardtii*.

### Literature:

[1] Zhao et al. (2004) Cell 3, 815-825.

[2] Iliev et al. (2006) Plant Physiol. 142, 797-806.

## Characterization of novel chemotactic factors for $\gamma\delta$ T-lymphocytes

[Krisztina Truta-Feles](#), Lagadari M., Lehmann K., Johannes Norgauer, Clinic of Dermatology, FSU

The recruitment of leukocyte populations to an area of inflammation is one of the most fundamental processes of immune reactivity, yet a number of the mechanisms which are important to this process are not clearly understood. A numerically small subset of human T lymphocytes expresses  $\gamma\delta$  TCR, a clonally distributed T cell receptor (TCR). Although TCR is a hallmark of adaptive immunity,  $\gamma\delta$  T-cells are classically considered as innate-like effectors. They share certain effector functions with  $\alpha\beta$  T cells as well as with NK cells and NKT cells like the ability to produce inflammatory cytokines involved in protective immunity against intracellular pathogens and tumours and to display strong cytolytic as well as bactericidal activities. Most  $\gamma\delta$  T cells can recognize ligands which are fundamentally different from the short peptides that are seen by  $\alpha\beta$  T cells in the context of MHC class I or class II molecules.

In order to investigate further physiological characteristics of  $\gamma\delta$  T cells, human peripheral blood mononuclear cells (PBMC) isolated  $\gamma\delta$  T cells were exposed to classical chemotactic factors such as N-formyl-methionyl-leucyl-phenylalanine (fMLP), complement factor 5a (C5a) and the endogenous mediator, histamine. Ligation of fMLP, C5a or histamine to its specific G-protein coupled cell surface receptors triggers different cascades of biochemical events, eventually leading to cellular activation.

Functional studies like migration assays, actin polymerisation measurements, intracellular calcium mobilization experiments and cytotoxicity assays suggests that fMLP, C5a and histamine have important biological effects on human  $\gamma\delta$  T lymphocyte activities.

### Literature:

- 1) Maghazachi A.A. (2000): Intracellular signalling events at the leading edge of migrating cells *The Int. J. of Biochem.* 32:931-943.
- 2) Born W.K., Reardon C.L., O'Brien R.L. (2006): The function of  $\gamma\delta$  T cells in innate immunity *Current Opinion in Immunology* 18:31-38.
- 3) Glatzel A., Wesch D., Schiemann F., Brandt E., Janssen O., Kabelitz D. (2001): Patterns of chemokine receptor expression on peripheral blood  $\gamma\delta$  T lymphocytes: strong expression of CCR5 is a selective feature of V $\delta$ 2/V $\gamma$ 9  $\gamma\delta$  T cells *The Journal of Immunology* 168:4920-4929.
- 4) Wittmann S., Fröhlich D., Daniels S. (2002): Characterization of the human fMLP receptor in neutrophils and in *Xenopus* oocytes *British J of Pharmacology* 135: 1375-1382..
- 5) Idzko M., la Sala A., Ferrari D., Panther E., Herouy Y., Dichmann S., Mockenhaupt M., Di Virgilio F., Girolomoni G., Norgauer J. (2002): Expression and function of histamine receptors in human monocyte derived dendritic cells *J.Allergy Clin Immunol* 109:839-46.

## Characterization of CaCRASP-2, a novel surface protein of *Candida albicans* which mediates immune evasion and adhesion to host cells

[Shanshan Luo](#)<sup>1</sup>, Uta-Christina Hipler<sup>2</sup>, Peter F. Zipfel<sup>1</sup>

<sup>1</sup> Dept. Infection Biology, HKI

<sup>2</sup> Clinic of Dermatology and Allergology, FSU

*Candida albicans* is an opportunistic host pathogen that can cause mucosal infections in healthy individuals, as well as life-threatening infections, especially in immunocompromised patients. This pathogenic yeast utilizes host complement regulators such as Factor H, Factor H like protein-1 (FHL-1) and C4BP for immune evasion. Here we identify a new Factor H binding protein of *C. albicans* upon screening of a cDNA expression library, which mediates immune evasion. Consequently this *C. albicans* complement regulatory-acquiring surface protein (CaCRASP-2) was recombinantly expressed and purified. Recombinant CRASP-2 binds the host complement regulators Factor H, FHL-1 and plasminogen. Attached to CRASP-2, all three host proteins are functionally active. Specific CRASP-2 antiserum was generated and used to demonstrate surface expression of CRASP-2 on *C. albicans* yeast and hyphal cells. CRASP-2 expression is up-regulated upon *C. albicans* switching from yeast to hyphae growth. In addition, CRASP-2 is also released and was identified in the culture medium. Soluble CRASP-2 binds back to the surface of *C. albicans*, and also binds to host cells. In the medium, the soluble CRASP-2 enhances cofactor activity of Factor H and assists *C. albicans* adhesion to host cells. In addition, expression and sequence variations of CRASP-2 was analyzed in clinical strains isolated from infected patients. CRASP-2 expression levels were either comparable to that of the wild type strain SC5314 or even higher. In addition, the nucleotide sequence is changed at 16 positions, and seven changes also affect the amino acid sequence. Thus, CaCRASP-2 represents a multifunctional virulence factor of *C. albicans*, which functions at different levels: (i) on the surface of *C. albicans*, CRASP-2 acquires host regulators of the complement and fibrinolytic system in order to control complement attack, homeostasis and tissue integrity, (ii) as a released protein, CRASP-2 binds back to the surface of the hyphal cells and also enhances complement inactivation in fluid phase, which results in reduced complement attack, (iii) upon binding to the surface of the host cells, CRASP-2 assists *C. albicans* adhesion to host cells.

## Diversity of natural endophytic bacteria in *Nicotiana attenuata* roots depends on plant genotype and soil type

[Hoang Hoa Long](#), Ian T. Baldwin, Department of Molecular Ecology, MPI CE

The wild tobacco, *N. attenuata*, native to the Great Basin Desert, USA, has evolved to germinate in post-fire nitrogen rich soils which are ideal for bacterial growth and root colonization. Yet the occurrence of endophytic bacteria and their interactions with their natural host have not been documented. In this study, we examined the diversity of culturable bacterial endophytes from the wild type (wt) and mutant *N. attenuata* plants that had been grown in 4 different soils collected from native habitats of *N. attenuata* in Utah, USA. The mutant plants were deficient in ethylene production (*irACO1*) and ethylene perception (*etr1*). A significant difference in colony forming unit (cfu) was found between the roots of wt and of mutant plants. Two-way ANOVA showed a significant effect of plant genotype and soil type on the cfu. In total, 140 bacterial isolates were collected. Among these isolates, 37 operational taxonomic units were identified based on the similarity of HinfI-digested 16S rDNA banding profiles by amplified ribosomal DNA restriction analysis (ARDRA). An equal proportion of isolates from wt and mutant plants was selected for 16S rRNA sequencing. The sequence analysis revealed that *Bacillus* sp. and *Pseudomonas* sp. were the most abundant genera isolated from wt and mutant plants while *Sphingobium* sp., *Sphingomonas* sp. and *Curtobacterium* sp. were exclusively found in wt plants. Three bacterial genera were found in *etr1* roots, four in *irACO1* roots, and six in wt roots. This study provides the first comprehensive information about the diversity of bacterial endophytes in *N. attenuata* roots and demonstrates that ethylene signaling pathway can influence this diversity.

**Growth inhibition of ecologically relevant bacterial species by the green alga, *Dictyosphaeria ocellata*.**

[Jennifer Sneed](#), Georg Pohnert, Inst. for Inorganic and Analytical Chemistry, Bioorganic Analytics, FSU

While interactions between microbes and sessile marine organisms such as sponges and corals have been well investigated, little is known about the interactions between macroalgae and the microbial community. Like sponges and corals, macroalgae are constantly exposed to large numbers of microorganisms, some of which are potentially pathogenic. Therefore, it is probable that macroalgae face selective pressure to regulate the surrounding microbial community. *Dictyosphaeria ocellata* is a green alga that grows in intertidal areas adjacent to mangrove forests and is therefore subject to heavy bacterial loads in the water. However, the surface of this alga remains relatively clean when compared to other co-existing abiotic and biotic surfaces. In this study we investigated the interaction between *D. ocellata*, and various strains of common marine bacteria in a laboratory culture. We found that the presence of the alga inhibits the growth of bacteria in culture. However, neither waterborne compounds exuded by the alga nor algal extracts produced similar effects. Our results suggest that *D. ocellata* does have a mechanism for regulating the surrounding microbial community, but that it may be related to factors other than constitutively released inhibitory chemicals.

Plenary Lecture **Molecular dissection of the *Colletotrichum* infection**

[Holger Deising](#)

Martin-Luther-Universität Halle-Wittenberg, Inst. für Agrar- und Ernährungswissenschaften,  
Phytopathologie und Pflanzenschutz

## Identification and characterisation of infection-associated genes in *Candida albicans*

[Francois Mayer](#), Duncan Wilson, Bernhard Hube, Dept. Microbial Pathogenicity Mechanisms, HKI

Our group has performed transcriptional profiling for different types and models of infection with *Candida albicans*. Samples from the oral cavity of HIV-positive patients, a model based on reconstituted human oral epithelium, infected human blood and infected liver from mice were analysed for genome-wide gene expression of *C. albicans*. Analysis of the obtained transcriptome data revealed a high number of significantly upregulated genes during the infection process. Besides some previously described and now well characterised infection-associated genes, a large percentage of genes encoded proteins with unknown functions. We hypothesised that a significant portion of these upregulated genes might play an important role in the initiation and persistence of *C. albicans* infections. *In silico* analysis identified a set of 50 genes with a high probability of involvement in the infection process. These were chosen for further characterisation and we have begun to systematically disrupt each of these genes. Our gene disruption strategy is based on a rapid PCR method (Gola *et al.*, 2003) using the strain BWP17, which is auxotrophic for arginine, histidine and uridine. First, ARG4- and HIS1-disruption cassettes are generated using primers containing approximately 100 bp homology to the immediate upstream and downstream sequences of the relevant target gene. Using the ARG- and HIS-disruption cassettes, both alleles of the target gene are deleted and the remaining uridine auxotrophy of the homozygous mutant can be used to restore a wild type copy of the gene of interest, thus satisfying Molecular Koch's postulates. More than 15 homozygote and several heterozygote mutants have already been obtained. Some of the mutants obtained were shown to have reduced adhesion and/or invasion and tissue damage properties. The phenotypes of selected mutants will be presented.

## Role of PARP-1 cleavage in *Chlamydia trachomatis*-induced host cell death

[HanXing Yu](#), Eberhard Straube, Jürgen Rödel, Institute for Medical Microbiology, FSU

*Chlamydia* has evolved an anti-apoptotic activity to evade host defense and induced cells damage to complete its infection cycle. However, factors and the precise mechanisms by which *Chlamydia* interfere with host cell death remain unclear. Here we proved that *Chlamydia trachomatis* infection induced necrotic like cell death. A pro-apoptotic Bcl-2 family protein, Bax, was activated and translocated to mitochondria after infection, without stimulating mitochondrial damage and matrix proteins release. Following apoptotic stimuli, effector caspase-3 was activated to cleave the 113 kDa Poly (ADP-ribose) polymerase (PARP-1) in fragments of 89 kDa and 24 kDa, which is a hallmark of apoptosis. During infection, no caspase-3 activation was detected; however, after treatment by death stimuli staurosporine, the activated caspase-3 and the subsequent apoptosis displayed in uninfected cells were inhibited by chlamydial infection. Interestingly, PARP-1 was cleaved to a multi-fragment form independent of caspase-3 activation in infected cells, which represented to different domains of this protein. Nicotinamide, a potential inhibitor of PARP-1 activity, efficiently decreased host cell death induced by *Chlamydia trachomatis*; on the other hand, it also blocked the reticulate bodies' division into elementary bodies, which demonstrated that as a DNA repair enzyme, PARP-1 also acted on the process of bacterial DNA replication to complete chlamydial development cycle. Chlamydial but not host cell protein synthesis contributed to this PARP-1 cleavage. Cell free degradation assay confirmed that this proteolytic activity only existed in cytosolic extraction of infected cells. The purified protein after column chromatography exhibited the 35 kDa and 29 kDa fragments by comas staining, 2-D gel electrophoresis proved that the 29 kDa fragment corresponded to the NH<sub>2</sub>-terminal portion of chlamydial proteolytic activity factor (CPAF). Our results gave the convincing evidence that intracellular bacterium *Chlamydia trachomatis* secreted its specific protein into host cell cytosolic to degrade host cell proteins so that it could efficiently regulate host cells death pathway to fulfill its own infection cycle and evade host defense.

## **Apoptosis inhibition of alveolar macrophages upon interaction with conidia of *Aspergillus fumigatus*.**

[Katrin Volling](#), Hanspeter Saluz, Dept. Cell and Molecular Biology, HKI

The opportunistic pathogen *Aspergillus fumigatus* (Af) causes the majority of cases of invasive aspergillosis (IA). Because Af enters the body through inhalation of air-borne conidia, the interaction of conidia with the innate immune system (alveolar macrophages) plays a key role in the aetiology of IA. The mechanisms underlying the anticonidial activity of macrophages and the relative resistance of conidia against the respective effector processes are a matter of debate. Modulation of host cell apoptosis has been reported to be one of the mechanisms pathogens employ to overcome host cell defences.

Hence, we focused our attention on the influence of Af conidia on staurosporine (STS)-induced apoptosis of murine alveolar macrophages (MH-S). MH-S cells exposed to Af conidia and treated with STS showed a decreased number of apoptotic cells compared to STS-induced control cells examined by flow cytometry analysis, DNA fragmentation and immunoblotting. The observed anti-apoptotic effect of Af conidia on MH-S cells was found to be associated with a significant reduction of active caspase-3, -6, -7, -8 and -9, which are critical mediators of receptor-mediated as well as mitochondria-mediated events of apoptosis. Furthermore, MH-S cells exposed to Af conidia showed a decreased level of cytosolic cytochrome c after apoptosis induction. An Af mutant strain lacking a specific enzyme in the dihydroxynaphthol (DHN)-melanin biosynthesis pathway (pksP), which is required for the biosynthesis of the gray-green pigment, had no inhibitory effect on STS-induced apoptosis. To characterize the cellular response of macrophages to wild type versus pksP mutant conidia, we investigated the alterations of proteins with two-dimensional gel electrophoresis (2-DE) followed by identification with a MALDI-TOF/TOF device. The most prominent changes were observed in glycolytic enzymes and proteins involved in maintenance of cellular integrity and NO production. Notably, comparison of WT versus pksP mutant infected MH-S cells revealed 8 proteins unique to WT, and 4 proteins unique to pksP infected macrophages. Our results indicate that an intermediate or derivative of DHN melanin might function as a virulence factor by enhancing the resistance of fungal cells against attack by host effector mechanisms but also has the ability to modulate host cell apoptosis.

## **Analysis of proteins secreted by the Dermatophyte fungus *Arthroderma benhamiae***

[Christoph Heddergott](#), Johannes Wöstemeyer, Inst. of General Microbiology and Microbial Genetics, FSU

Dermatophytes are ascomycetous fungi specialized on degradation of keratinized tissue like hair, nails and skin. The zoophilic species *Arthroderma benhamiae* infects animals (e.g. guinea pigs) as well as humans and induces an inflammatory host response while being eliminated comparatively fast by its defence systems. The fast growing and well cultivable species is a model organism for elucidating general pathogenicity mechanisms and factors which specify the different host adaptation levels observed among Dermatophyte species. Here we characterise this interaction by investigating proteins which are secreted and therefore directly in contact with the host tissue.

For secretome analysis, protein samples from culture supernatants and LiCl cell wall extracts obtained under various culture conditions were separated by two-dimensional polyacrylamide gel electrophoresis and identified by mass spectrometry. *Arthroderma benhamiae* is able to grow on keratin as the sole carbon/nitrogen source. During growth on this substrate, protease secretion was strongly induced compared to conditions when monomers were fed. Beside this, numerous secreted proteins involved in carbon hydrate and lipid metabolism as well as functionally uncharacterised proteins were identified. The comparison of non-covalently cell wall associated proteins released by LiCl treatment and those from the culture fluid revealed different affinities of proteins to the cell wall matrix after secretion. Furthermore, rapid degradation of several secreted protein exerts considerable influence on protein patterns.

This study presents the first comprehensive analysis of the *A. benhamiae* secretome, including insights into time and spatial distribution of proteins. Prospective studies will focus on the characterisation of specific proteins from the secretome, especially on those which do not contain conserved structures and are yet undescribed, but possibly exert influence on the infection process.

## **Real Time Monitoring of Secondary Metabolite Gene Expression in *Aspergillus nidulans***

[Anindita Sarkar](#), Uwe Horn, Bio Pilot Plant for Natural Products, HKI

The burgeoning class of multi drug resistant Micro-organisms and escalating figures of chronic diseases have generated the need for new drugs. Discovery of life saving drugs from filamentous fungi viz Penicillin, Lovastatin, Cephalosporin etc has highlighted importance of filamentous fungi as a promising repository of natural products. Moreover nearly 40 putative secondary metabolite gene clusters have been speculated in Genetic model organism *Aspergillus nidulans*. This is an indication that more secondary metabolites could be discovered from this fungus.

When faced with enduring conditions, to gain an edge over competitor species, microbes synthesize secondary metabolites. Depleting nutrient sources pose one such enduring situation. We have done time chase experiments under single nutrient depletion conditions and shown the expression profile of a putative secondary metabolite gene cluster.

## Lignin degradation: From aerobic fungal halogenation to anaerobic bacterial dehalogenation

[Lidan Ye](#), Anke Schilhabel, Jörg Nüske, Gabriele Diekert, Inst. of Applied and Ecological Microbiology, FSU

White-rot fungi are known to degrade lignin by means of different peroxidases, producing halogenated aromatic compounds and phenyl methyl ethers, while several strictly anaerobic bacteria belonging to the genus *Desulfitobacterium* are able to reductively dechlorinate chlorinated aromatic or aliphatic compounds as well as demethylate phenyl methyl ethers. This study is aimed to elucidate the interaction between the aerobic fungi and the anaerobic bacteria in the formation and degradation of halogenated and/or methylated compounds in lignin decomposition.

In this project, we studied fungal halogenation, targeting at the production of organohalogens and especially methoxylated organohalogens, both in artificial medium and on natural substrates. Although the ubiquitous capacity of producing halogenated aromatic compounds among basidiomycetes has been well studied, the enzyme or the process responsible for the halogenation is not yet clear. In this study, we have detected versatile peroxidases from the white rot fungus *Bjerkandera adusta* that might be involved in this process, as they displayed haloperoxidase activity besides their ligninolytic enzyme activities. In cultures with haloperoxidase activity halogenated products such as 3-chloro-4-methoxybenzaldehyde were formed.

In the second part of this project, we focused on the anaerobic dechlorination and debromination. Surprisingly, tetrachloroethene (PCE) dechlorinating anaerobes producing *cis*-1,2-dichloroethene as end product of PCE dechlorination also dehalogenated the dibromoethene, which was further dehalogenated to vinyl bromide (and ethene) by dehalogenases isolated from *Desulfitobacteria* or *Sulfurospirillum multivorans*. This finding indicates that bromine is more easily eliminated from halogenated compounds than chlorine substituents. This finding might lead to the conclusion that an interaction between bromination, which is the preferred halogenations process mediated by fungi, may occur with debrominating anaerobes in nature.

To check for the possibility of the involvement of anaerobic dehalogenating bacteria in lignin degradation, studies were carried out both *in vitro* and *in vivo*. Forest soil samples were checked for their ligninolytic activity and anaerobic *o*-demethylation/dehalogenation potential. In these samples, both activities were detected. In addition, *o*-methylated/halogenated fungal metabolites were fed to dehalogenating anaerobic bacteria. For these studies, a method for co-cultivation of aerobic fungi and anaerobic bacteria was established.

The initial results pointed to a possible halogen cycle between aerobic fungi and anaerobic bacteria which might play a role in lignin degradation.

**Genomic studies of the endofungal, rhizoxin producing bacterium *Burkholderia rhizoxinica*.**

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The recently discovered bacterial species *Burkholderia rhizoxinica* and *B. endofungorum* are intracellular endosymbionts of the plant pathogenic fungus *Rhizopus microsporus*, which can cause a severe plant disease called rice seedling blight. Formerly it was assumed that the pathogenicity factor rhizoxin is produced by the fungus itself. However, it was shown that the bacterial endosymbiont is responsible for the toxin formation. As a solid basis to investigate the endofungal lifestyle and evolution of this intracellular toxin factory the genomes of both type strains were sequenced. Multiple genes and gene clusters were identified that might play a role in fungal-bacterial interaction. In order to prove the involvements of candidate genes in host-symbiont communication, a system for genetic engineering of the strains is being developed.

## Real-time PCR for detection of pheromone and receptor gene expression in the basidiomycete *Schizophyllum commune*

[Susann Erdmann](#), E. Kothe, Inst. of Microbiology – Microbial Phytopathology, FSU

The sexual development of the heterothallic basidiomycete *Schizophyllum commune* depends on a tetrapolar mating system. A mating type specific pheromone receptor, belonging to the G-protein coupled, seven transmembrane domain receptors, interacts with several pheromones, small lipopeptides, and distinguishes between self and non-self. The importance of the pheromone/receptor system in *S. commune* is well-investigated, however there are little information about the expression of mating genes. By means of real time PCR the expression level of a pheromone and a receptor gene was measured and quantified relative to the expression of reference genes. The expression level of *bar2* receptor gene and *bap2(2)* pheromone gene was determined in wildtype mating 12-43 ( $A_{3,5}B_{2,2}$ , *ura1*<sup>-</sup>) x 4-39 ( $A_{1,1}B_{3,2}$ ) over a 72-hour time period. The quantification of mating genes was also performed in mating with a receptor transformant strain. These results are the first information about the expression of mating specific genes in the filamentous fungi *S. commune*.

## **Regulation of Polyketide Synthase Gene Clusters in *Aspergillus nidulans***

[Alexander Funk](#), Uwe Horn, Bio Pilot Plant for Natural Products, HKI

The availability of fully sequenced fungal genomes has opened the doors to a wealth of information on putative secondary metabolite gene clusters. Genome analyses showed that up to 30 clusters of polyketide synthase (PKS) genes exist in *Aspergillus nidulans* yet much is not known about their regulation and the formed metabolites.

Weak promotor activity and short time of expression make the gene regulation studies challenging. We use a reporter system based on the marine copepod *Gaussia princeps* luciferase successfully applied in *A. nidulans*. The luciferase also contains a secretion signal facilitating extracellular signal detection during various cultivation techniques.

## Heavy metal ion stress – Induction of chemical differentiation in *Streptomyces coelicolor*

[Anne Behrend](#), Dieter Spittler, Wilhelm Boland, Dept. of Bioorganic Chemistry, MPI CE

Microorganisms from the genus *Streptomyces* are well known for their highly diverse secondary metabolism which makes them a prominent source of bioactive substances in particular polyketides and non-ribosomal peptides. In contrast to their pharmaceutical value the ecological role of such secondary metabolites for the producing organism in its natural environment is little studied.

A large number of polyketide- and non ribosomal peptide-gene clusters exist whose products are unknown. These so called “sleeping genes” are not expressed under standard growth conditions but may become functional under stress in order to allow physiological adaptation to specific environmental conditions.

To study the impact of stress factors on *Streptomyces* the model organism *Streptomyces coelicolor* was treated with heavy metal-ions. In particular  $\text{Co}^{2+}$ -ions induced drastic alterations leading to a large diversity of phenotypes with varying pigmentation (red, blue, black). These dramatic metabolic changes are reflected in both the changes of coloured secondary metabolite production and the volatile profile. So far the volatile pattern of all phenotypes was monitored. The identity of the compounds was proven for example by overexpression of the isozizaene synthase.

Attempts to isolate and identify the secondary metabolites responsible for the pigments clearly reveal that a large variety of orange, purple and red coloured secondary metabolites are formed by the red phenotype.

Besides the direct identification of such induced secondary metabolites the adaptation of the phenotypes to heavy metal ion stress is addressed by microarray studies to reveal the genome wide alteration of gene expression. Validation of RNA preparation showed that certain RNAs are under-represented in the in the red coloured phenotype.

## Poster

Christian Hummert

1. Optimal Probeset Reassembly
2. Improving the Quality of Microarray Analysis with Machine Learning Techniques

3. Lidan Ye

Reductive dehalogenation of dibromoethene by *Sulfurospirillum multivorans* and *Desulfitobacterium hafniense* PCE-S

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The reductive dehalogenation of halogenated aliphatic alkenes such as tetrachloroethene (PCE), under anaerobic conditions can be catalyzed by strictly anaerobic bacteria. The reductive dehalogenases of *Sulfurospirillum multivorans* [1] and *Desulfitobacterium hafniense* PCE-S [2] as well as several other bacterial dehalogenases are well characterized concerning their enzymatic properties and substrate spectra. The end product of PCE dehalogenation by both strains is *cis*-1,2-dichloroethene (cDCE).

Brominated alkenes were tested as substrates for the growth of both organisms and as substrate for anaerobic enzymatic dehalogenation. Interestingly, both strains were able to dehalogenate the brominated analogue of the end product of PCE dehalogenation, *cis*-1,2-dibromoethene (cDBE), as well as *trans*-1,2-dibromoethene (tDBE) during cultivation using pyruvate as electron donor. In cultures containing PCE plus DBE as electron acceptors, both organohalogenes were converted simultaneously. However, in these cultures inhibition of PCE dehalogenation by DBE and inhibition of growth was observed to different extents in both strains. *Sulfurospirillum multivorans* debrominated cDBE and tDBE to vinylbromide while *Desulfitobacterium hafniense* PCE-S was able to produce ethene as end product. It was tested if the debromination of DBE by *S. multivorans* and *D. hafniense* PCE-S is mediated by the known tetrachloroethene reductive dehalogenases or by separate enzymes. The kinetic properties of the dehalogenation of DBE as well as other brominated alkenes are reported.

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4. Susann Schindler<sup>1</sup>, Axel Brakhage<sup>2,3</sup>, Peter F. Zipfel<sup>1,3</sup>

***Arthroderma benhamiae* uses a dual strategy to evade host complement attack**

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Dermatophytes mediate human cutaneous mycosis. The immune evasion strategies of dermatophytes are currently unknown. Therefore we analyze immune evasion of the human pathogenic dermatophyte *Arthroderma benhamiae*, a teleomorph form of *Trichophyton mentagrophytes*. We investigate binding of human proteins to the surface of this pathogen. *Arthroderma benhamiae* binds human complement Factor H and the Factor H related protein 1 (CFHR1) to the surface as demonstrated by direct binding assays, immunostaining and ELISA. Human Factor H is an important complement regulator protein of the alternative pathway of complement activation and CFHR1 is member of the Factor H protein family. In addition we show binding of plasminogen, the key protease in fibrinolysis. Human Factor H,

CFHR1 and plasminogen is local expressed by human keratinocytes in skin, which is shown by Western Blot analyses of culture supernatants.

*Arthroderma benhamiae* acquires human complement regulators Factor H and CFHR1 to the surface to evade complement attack. The inactivation of C3b by pathogen bound Factor H and CFHR1 impeded the deposition of C3b on the surface of *Arthroderma benhamiae*. Thus the dermatophyte is prevented against opsonization by host complement.

In addition *Arthroderma benhamiae* utilizes an second independent strategy to inhibit human complement. The fungus secretes complement degrading proteases, which avoid complement activation. That way the dermatophyte *Arthroderma benhamiae* protects itself against complement attack.

5. V. Schroeckh, A. N. Funk, A. Sarkar, U. Horn, A. A. Brakhage

#### **REPORTER SYSTEM FOR MONITORING SECONDARY METABOLITE PROMOTOR ACTIVITY IN THE FILAMENTOUS FUNGUS *ASPERGILLUS NIDULANS***

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The availability of fully sequenced fungal genomes has opened the doors to a wealth of information on putative secondary metabolite gene clusters. These clusters often contain nonribosomal peptide synthetase and/or polyketide synthase genes. Since their biological function and regulation are unknown, these clusters often have been viewed as silent. Weak promoter activity and short time of expression make the gene regulation studies challenging. Here we present a reporter system based on the marine copepod *Gaussia princeps* luciferase successfully applied in fungal cells.

The enzyme exhibits a flash kinetics resulting in a short-lived intense signal, which makes detection at low expression levels possible. The codon usage is optimized for mammalian cells and has been successfully applied in *Aspergillus nidulans*. The luciferase also contains a secretion signal facilitating extracellular signal detection during various cultivation techniques.

6. Katharina Gropp, Michael Reuter, Gerhard D. Wieland, Christine Skerka and Peter F. Zipfel

#### **EGR and NF $\kappa$ B are signal transducer in macrophages upon stimulation with the human pathogenic yeast *Candida albicans***

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*C. albicans* is the major human opportunistic fungal pathogen that causes life-threatening systemic infections. Macrophages represent the first cellular defence line that can recognize yeast cells by TLRs and induce immune response by e.g. release of cytokines. EGRs (early growth response proteins) interact physically and functionally with the nuclear factor NF- $\kappa$ B and induce cytokine gene expression. As NF- $\kappa$ B proteins are key transcription factors in TLR-signaling we asked whether EGR proteins by interacting with NF- $\kappa$ B participate in nuclear response in macrophages to *Candida*.

RT-RNA analysis showed transcriptional activation of EGR-1, EGR-2, EGR-3 and EGR-4 as well as p65, p50 in macrophages exposed to the yeast- and hyphae-form of *Candida*. The yeast-form induces synthesis of pro-inflammatory cytokines like MIP-1 $\alpha$  and MIP-1 $\beta$  and hyphae induce the anti-inflammatory cytokine IL-10. Signal transduction pathways were characterized by specific inhibitors. The ERK inhibitor (PD98059) and NF- $\kappa$ B -pathway inhibitor (BAY11-7082) showed that yeast and hyphae utilized both pathways. With transfection assays and confocal microscopy we demonstrate that the synthesis of chemokines MIP-1 $\alpha$  and MIP-1 $\beta$  in macrophages in response to *Candida* is mediated by synergistic activity of EGR-2 and NF- $\kappa$ B. Yeast and hyphae forms of *C. albicans* bind to host macrophages via specific receptors, such as TLR2 and TLR4. Attachment of the yeast or hyphae initiates a signal cascade which induces the transcription factors EGR or NF-

kappaB. Following nuclear translocation, these factors induce transcription of immune effector genes, e.g. MIP-1 $\alpha$  and MIP-1 $\beta$ . Here we established a cell system to characterize signal cascades and transcription factors induced by *C. albicans*.

Members of the EGR and NF-kappaB family, EGR-2 and p65 genes are induced in human macrophages by *C. albicans*, form physical complexes and regulate chemokines expression.

## 7. Rainer Melzer and Günter Theißen

Protein-DNA interactions of floral homeotic transcription factors

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MADS-domain transcription factors act in flower development as homeotic selector proteins that determine floral organ identity. Although they are well understood genetically, the molecular mechanism of their function is still largely unclear. According to one widely discussed hypothesis, the 'floral quartet' model, the combinatorial formation of DNA-binding tetrameric complexes of MADS-domain transcription factors specifies floral organ identity. To critically test the floral quartet model, we studied protein-protein and protein-DNA interactions of floral homeotic proteins. Our findings have surprising implications for the DNA-binding specificity of floral homeotic proteins and the evolutionary origin of floral quartets.

## 8. Christina Große

Differences in the developmental cycle of *Chlamydia trachomatis* within epithelial cells and fibroblasts

*Chlamydia trachomatis* infection is a wide-spread cause of sexually transmitted and ocular diseases. Its growth is characterized by a unique developmental cycle in which non-infectious, metabolically active reticulate bodies (RBs) are formed intracellularly after infection of host cells with metabolically inert elementary bodies (EBs). After some rounds of multiplication RBs convert back to EBs and subsequently are released from the host cell. Chlamydia are able to persist inside a host cell by formation of enlarged, atypical RBs that do not undergo cell division and conversion to EBs. This persistence is considered to be the cause of chronic *C. trachomatis* infection. In contrast to epithelial cells that support a productive lytic chlamydial growth cycle, fibroblasts may represent reservoirs of persisting chlamydia. Indeed atypical reticulate bodies have been found in this cell type in synovial biopsies of patients with reactive arthritis (1).

In this study we compared the infection of epithelial cells and fibroblasts with *C. trachomatis* to examine whether fibroblasts may support the development of intracellular persistence of the pathogen. Electron micrographs demonstrated that chlamydia within inclusions in fibroblasts exhibited the morphology of atypical RBs. Furthermore fibroblasts showed a dramatically reduced production of EBs compared to epithelial cells upon chlamydial infection. Interruption of the complete chlamydial growth cycle in fibroblasts was associated with a strong expression of indoleamine 2,3-dioxygenase (IDO) which usually results in depletion of cellular tryptophan and is considered to be one mechanism responsible for chlamydial persistence (2). Because IDO expression is induced by interferons (IFN) we examined if a soluble factor is involved in the inhibition of EB differentiation. This was shown by culture supernatants harvested from infected fibroblasts that reduced the production of infectious chlamydia in epithelial cells. IDO expression in fibroblasts may be mainly induced by IFN- $\beta$  which was detected by ELISA in supernatants of fibroblasts but not of epithelial cells. Surprisingly, 1-methyltryptophan (1-MT), an inhibitor of IDO activity, had no significant effect on the infectious progeny of chlamydia in fibroblasts, indicating that other mechanisms must be involved in growth cycle arrest.

In conclusion this work shows that *C. trachomatis* undergoes an altered developmental cycle within fibroblasts which results in a blockade of the differentiation of RBs into EBs. In further experiments we will investigate chlamydial genes differentially up-regulated in persisting RBs

in fibroblasts and possible differences in the maturation of *Chlamydia*-containing endosomes within fibroblasts and epithelial cells.

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**9. Dorit Schmidt** ILRS

**10. Carsten Thoms** JSMC

#### **11. Christoph Heddergott**

*In-vitro* analysis of proteins secreted by *Arthroderma benhamiae*

#### **12. Krisztina Truta-Feles**

##### **Characterization of histamine receptor functions in $\delta\gamma$ T lymphocytes**

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Histamine ( $\beta$ -imidazoleethylamine) is an endogenous mediator involved in various responses of immune and non-immune cells. It is stored in the granules of tissue mast cells and blood basophils, and is released upon activation by IgE crosslinking<sup>1</sup>. Histamine receptors are expressed on the surface of in human monocyte derived dendritic cells<sup>2</sup>, eosinophils<sup>3</sup>, mast cells<sup>4</sup>, T cells, keratinocytes<sup>5</sup>, and NK cells<sup>6</sup>. Beyond its physiological functions, histamine was described as an autocrine and paracrine/exogene growth factor for malignant melanomas and leukemic cells. Histamine and histidine decarboxylase (HDC), the only enzyme that catalyzes histamine production, has been proved to be present in elevated concentration in proliferating tissues, including tumor cells<sup>7</sup>.

The recruitment of leukocyte populations to an area of inflammation is one of the most fundamental processes of immune reactivity, yet a number of the mechanisms which are important to this process are not clearly understood.

A numerically small subset of circulating human T lymphocytes expresses  $\gamma\delta$  TCR, a clonally distributed T cell receptor (TCR). Most  $\gamma\delta$  T cells can recognize ligands which are fundamentally different from the short peptides that are seen by  $\gamma\delta$  T cells in the context of MHC class I or class II molecules<sup>8</sup>.

In order to investigate further physiological characteristics of  $\gamma\delta$  T cells, human peripheral blood mononuclear cells (PBMC) isolated  $\gamma\delta$  T cells were exposed to the endogenous mediator, histamine. Ligation of histamine to its specific G-protein coupled cell surface receptor, triggers different cascades of biochemical events eventually leading to cellular activation.

Functional studies suggest that histamine has important biological effects on human  $\gamma\delta$  T lymphocyte activities.

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