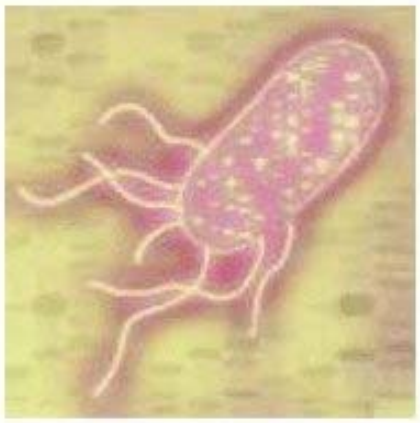

ERA-NET PathoGenoMics

Proceedings of the

Joint Annual Seminar on the Projects of the 1st and 2nd calls of the ERA-NET PathoGenoMics

January 18-19, 2010
Costa Adeje, Tenerife,
Canary Islands, Spain

Welcome

Welcome to the Joint Annual Seminar on the Projects of the 1st and 2nd calls of the ERA-NET PathoGenoMics. The seminar takes place in the beautiful island of Tenerife, Canary Islands.

The programme consists of talks and poster presentations of the two calls' research projects and research findings achieved in those. This seminar enables the participants to get an overall picture of the research undertaken in this ERA-NET. We hope that the seminar also facilitates strengthening the existing research contacts and creation of new ones.

The funding period for the 1st call's projects is Feb. 2007 – Jan. 2010. Thus, this will be the last seminar for the projects of the 1st call and this seminar will be a part of the final evaluation of those. The funding period for the 2nd call's projects it is Feb. 2009 – Jan. 2012.

On behalf of the ERA-NET PathoGenoMics,
the organizing team:

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CONTENT

PROGRAMME

Sunday, January 17, 2010

09.00 – 11.00 Registration at the hotel lobby

11.00 – 15.00 Social programme: Gathering at the hotel lobby at 11.00, transportation to Adeje and a short visit to the town; folkloric music and dancing; lunch; transportation back to the hotel. - Free of charge to all participants and their family members. All courtesy of the City Hall of Adeje.

17.30 – 19.00 Registration at the hotel lobby

Monday, January 18, 2010

8.00 - 9.00 Registration in front of the seminar room *(ground floor)*

9.00 - 9.30 **Welcome and opening of the seminar**
José Miguel Rodríguez Fraga, Major of Adeje
Marion Karrasch, Coordinator of the ERA-NET PathoGenoMics

Joint calls of the ERA-NET PathoGenoMics
Julio Barbas, Ministry of Science and Innovation, Spain

SESSION 1. 1st call projects
Chair: Wolf-Dietrich Hardt, Germany *(member of the Scientific Advisory Board)*

9.30 - 10.15 **SPATELIS: Spatio-temporal analysis of Listeria-host protein interactions**
Project Coordinator: Trinad Chakraborty, Germany

10.15 – 11.00 **RNAi-Net: A global RNAi approach to unravel eukaryotic host functions that modulate bacterial infections**
Project Coordinator: Thomas F. Meyer, Germany
- Christian Frank, Germany

11.00 - 11.30 Coffee at the terrace

SESSION 2. 1st call projects
Chair: Wolf-Dietrich Hardt, Germany *(member of the Scientific Advisory Board)*

11.30 - 12.15 **Deciphering the intersection of commensal and extraintestinal pathogenic E. coli**
Project Coordinator: Ulrich Dobrindt, Germany

12.15 – 13.00 **HELDIVNET: Parasite and host genetic diversity in Helicobacter infections**
Project Coordinator: Sebastian Suerbaum, Germany
- Rainer Haas, Germany
- Christine Josenhans, Germany

13.00 - 15.00 Lunch in the hotel restaurant

SESSION 3. 1st call projects
Chair: Matti Sarvas, Finland *(member of the Scientific Advisory Board)*

15.00 – 15.45 **Glycoshield: Surface modulation of the fungal & host response using a genomic approach**
Project Coordinator: Jesus Pla, Spain

15.45 - 16.30 **Large scale screening of potential key factors involved in the commensalism / virulence transition of Enterococcus faecalis**
Project Coordinator: Axel Hartke, France
- Johannes Huebner, Germany

16.30 - 17.30 **POSTER SESSION** and coffee

SESSION 4. 1st call projects
Chair: Matti Sarvas, Finland *(member of the Scientific Advisory Board)*

17.30 - 18.15 **FunPath: Genomic approaches to unravel the molecular mechanisms of pathogenicity in the human fungal pathogen Candida glabrata**
Project Coordinator: Karl Kuchler, Austria
- Tobias Schwarzmueller, Austria
- Ekkehard Hiller, Germany
- Sascha Brunke, Germany

18.15 – 19.00 **ECIBUG: European initiative to fight Chlamydial infections by unbiased genomics**
Project Coordinator: Matthias Maass, Austria
- Agethe Sbutil, France
- Mirja Puolakkainen, Finland
- Georg Wick, Austria

21.00 - Conference Dinner in the restaurant *La Vieja*

Tuesday, January 19, 2010

SESSION 5. 1st call projects
Chair: Herve Bercovier, Israel *(member of the Scientific Advisory Board)*

9.00 – 9.45 **EPS-Matrix: Exploring protein secretion within the bacterial biofilm matrix**
Project Coordinator: Jean-Marc Ghigo, France

9.45 – 10.30 **Pneumocystis pathogenomics: Unravelling the colonization-to-disease shift**
Project Coordinator: Eduardo Dei-Cas, France
- Enrique Calderon, Spain

10.30 - 11.30 **POSTER SESSION** and coffee

SESSION 6. 1st call projects
Chair: Herve Bercovier, Israel *(member of the Scientific Advisory Board)*

11.30 - 12.15 **A comparative molecular analysis of GAS and GBS pathogenesis**
Project Coordinator: Patrick Trieu-Cuot, France
- Emanuel Hanski, Israel
- Philippe Glaser, France

12.15 – 13.00 **Systematic analyses of kinase and phosphatase function in morphological, environmental and virulence responses of the human fungal pathogen Candida albicans**
Project Coordinator: Robert Arkowitz, France
- Daniel Kornitzer, Israel
- Joachim Morschhäuser, Germany

13.00 - 15.00 Lunch in the hotel restaurant

15.00 – 17.00 **SESSION 7. 2nd call projects**
Chair: Guido Grandi, Italy *(member of the Scientific Advisory Board)*

15.00 – 15.15 **Genome wide screening of the human pathogen Neisseria meningitidis for proteins enhancing serum resistance and evaluation of their vaccine potential**
Project Coordinator: Ulrich Vogel, Germany

15.15 – 15.30 **Functional genomics of host-pathogen interactions using high-throughput screenings: a novel approach towards identifying therapeutic/prophylactic targets**
Project Coordinator: Jose Antonio Bengoechea, Spain

15.30 – 15.45 **Pathogenomic approach to explore the use of bacterial interference as alternative treatment of recurrent urinary tract infections**
Project Coordinator: Ulrich Dobrindt, Germany

15.45 – 16.00 **ADHRES-Signature Project**
Project Coordinator: Sophie de Bentzmann, France

16.00 – 16.15 **sncRNAomics: High throughput comparative sncRNAome analysis in major Grampositive human pathogenic bacteria: functional characterisation by a systems biology approach and peptide nucleic acid drug design**
Project Coordinator: Torsten Hain, Germany

16.15 – 16.30 **TRANSPAT: Transcriptional networks controlling virulence in filamentous fungal pathogens**
Project Coordinator: Antonio Di Pietro, Spain

16.30 – 16.45 **The cell wall as a target to improve antifungal therapy against Aspergillosis**
Project Coordinator: Axel A. Brakhage, Germany

16.45 – 17.00 **Pathogenomics of increased Clostridium difficile virulence**
Project Coordinator: Maja Rupnik, Slovenia

17.00 – 17.30 Coffee at the terrace

SESSION 8. 2nd call projects
Chair: Guido Grandi, Italy *(member of the Scientific Advisory Board)*

17.30 – 17.45 **ChlamyTrans: Transcriptome-based monitoring and eradication of chronic Chlamydial infection**
Project Coordinator: Matthias Maass, Austria

17.45 – 18.00 **Mechanisms and modulation of innate immune responses to Streptococcus pneumoniae and S. pyogenes**
Project Coordinator: Pavel Kovarik, Austria

18.00 – 18.15 **Pathomics: Host-pathogen protein-protein interactions and their influence on the host metabolome**
Project Coordinator: Thomas Rattei, Germany

18.15 – 18.30 **METAGUT: Development, prevention and early diagnostic detection of Clostridium difficile associated colitis**
Project Co-coordinator Vitor Martins dos Santos, Germany

18.30 - 18.45 **Identification of hot spots of divergence and rapidly changing genes within Shiga toxin -producing Escherichia coli**
Project Coordinator: Alexander Mellmann, Germany

18.45 – 19.00 General discussion

20.00 - Cocktail/dinner in the hotel garden

LIST OF POSTERS

	LAST NAME	FIRST NAME	Country	Project	Call	Title
1	**Emödy**	Levente	Hungary	E.coli	1st	Non-immune and immune haemolysis may both contribute to disease progression in infection by alpha-haemolysin producing *Escherichia coli*
2	**Peter**	Harald		E.coli	1st	DNA Microarray for the Phylogenetic Classification of Pathogenic Escherichia coli Strains
3	**Szíjártó**	Valéria	Hungary	E.coli	1st	Lack of major immune-determinants on enteric bacteria results in enhanced cross-protective capacity of live attenuated mutants
4	**Zdziarski**	Jaroslaw	Germany	E.coli	1st	Host driven adaptation of asymptomatic bacteuria Escherichia coli strain 83972
5	**Toth**	Istvan	Hungary	E.coli	1st	Comparative genetic analysis of avian extraintestinal and intestinal Escherichia coli strains isolated in Hungary
6	**Salin**	Olli	Finland	ECIBUG	1st	Developing Electric Cell-Substrate Impedance Sensing -method to study Chlamydia pneumoniae infection in HL-cells.
7	**Garcia Martinez**	Mª Begoña	Spain	EPS-Matrix	1st	Protein-mediated biofilm development in Staphylococcus aureus.
8	**Brunke**	Sascha	Germany	FunPath	1st	Candida glabrata persistence and immune evasion strategies
9	**Hiller**	Ekkehard	Germany	FunPath	1st	Comprehensive gene deletion study to identify cell wall organisation and structure in Candida glabrata
10	**Schwarzmüller**	Tobias	Austria	FunPath	1st	Transcriptional rewiring of the Candida glabrata cell wall biosynthesis CBK1
11	**Brachhold**	Martina	Germany	Glycoshield	1st	Tsa1p modulates the cell surface of *Candida albicans*
12	**Cornu**	Amandine	France	Glycoshield	1st	Study of the Candida albicans IFF genes family during interactions with the host.
13	**Fradin**	Chantal	France	Glycoshield	1st	Transcriptional regulation of phosphopeptidomannan and phospholipomannan ?-1,2 mannosylation processes
14	**Herrero de Dios**	Carmen	Spain	Glycoshield	1st	Functional analysis of new elements of the SVG and HOG pathways in the fungal pathogen Candida albicans
15	**López**	M. Carmen	Spain	Glycoshield	1st	Phenotypic and transcriptional responses to protein N-glycosylation deficiency in Candida albicans
16	**Román González**	Elvira	Spain	Glycoshield	1st	Analysis of surface alterations in Candida albicans MAPK mutants
17	**Sarazin**	Aurore	France	Glycoshield	1st	Analysis of Candida albicans cell wall glycans during phagocytosis by macrophage
18	**Tielker**	Denis	Germany	Glycoshield	1st	Structure and function of the signaling mucin Msb2 in Candida albicans
19	**Rossi**	Mirko	Finland	HELDIVnet	1st	Genomics of non-Helicobacter pylori gastric Helicobacter species
20	**Friaza Patiño**	Vincente	Spain	*PNEUMOCY*	1st	Comparison of proteomic profiles in the Bronchoalveolar lavage fluid of Idiopathic pulmonary fibrosis patients with and without Pneumocystis colonization
21	**Martinez**	Anna	France	*PNEUMOCY*	1st	Pneumocystis carinii Cell and Biological Cycles: Ploidy and Trophic-to-Cystic Form Transition
22	**Bohn (1)**	Erwin	Germany	RNAi-NET	1st	Investigation of host cell requirements for Yop injection by Yersinia into host cells (Poster presentation)
23	**Bohn (2)**	Erwin	Germany	RNAi-NET	1st	Factors involved in Bartonella induced VEGF secretion
24	**Frank**	Christian G.	Spain	RNAi-NET	1st	Identification of Host factors affecting immune activation by Klebsiella pneumoniae
25	**Li**	Chun-Mei	Finland	RNAi-NET	1st	Development of Read-out system for RNAi screening of Yersinia-host interaction
26	**Martínez Soria**	Natalia	Spain	RNAi-NET	1st	RNAi-based screening of fibroblast functions that restrain Salmonella intracellular replication
27	**Fernando Mariscotti**	Javier	Spain	SPATELIS	1st	Characterization of new Listeria monocytogenes surface proteins of the LPXTG family
28	**Kärst**	Uwe	Germany	SPATELIS, RNAi	1st	Targeting the Met pathway activated by the invasin Internalin B from Listeria monocytogenes
29	**Bernard**	Christophe	France	ADHRESS	2nd	The "ADHRES Chip": differential expression of Pseudomonas aeruginosa genes involved in ADHesion and RESistance to antibiotics.
30	**del Mar Cendra**	Maria	Spain	Identification	2nd	Regulatory effect of H-NS in Escherichia coli ribonucleotide reductase genes

TALKS: 1st call projects

Name	**Trinad Chakraborty**
Organisation	Trinad.Chakraborty@mikrobio.med.uni-giessen.de
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Title of the project	SPATELIS: Spatio-temporal analysis of Listeria-host protein interactions
Title of your presentation	

Name	**Thomas F. Meyer**
Organisation	Max Planck Institute for Infection Biology
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Title of the project	Global RNAi approaches to unravel eukaryotic host functions that modulate bacterial infections (acronym: RNAi-NET)
Title of your presentation	The RNAi-Net – Insights into the role of host cell functions during the course of bacterial infections

The initiation and the progression of infections depend on specific molecular interactions between pathogens and the host. Conversely, inhibition of such interactions and signalling pathways might allow eliminating an infection. While investigations of virulence associated factors on the pathogen side have successfully progressed over years, a breakthrough for studying the role of host cell factors has been achieved recently with the discovery of RNA interference (RNAi). Within the umbrella of the ERA-NET PathoGenoMics, members of the RNAi-Net have started to systematically dissect important host cell functions playing roles in a variety of bacterial infections using the RNAi-based loss-of-function approach. This presentation will summarize major achievements of the RNAi-Net consortium during the past four years. Altogether, our findings support the idea of novel treatment options against both acute and chronic bacterial infections.

As an example, the analysis of host cell signalling routes exploited by intracellular Chlamydia species will be highlighted: An RNA-interference screen was conducted in human epithelial cells that led to the identification of numerous host factors influencing *C. trachomatis* replication in an either positive or negative fashion. Subsequent in-depth analysis points towards members of the MAP kinase signalling pathway as well as the Golgi apparatus as central host cell elements, crucial for the progression of *C. trachomatis* infections.

Name	**Ulrich Dobrindt**
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Title of the project	Deciphering the interface of commensal and extraintestinal pathogenic *E. coli*
Title of your presentation	Deciphering the interface of commensal and extraintestinal pathogenic *E. coli*

E. coli causing extraintestinal diseases (ExPEC – Extraintestinal Pathogenic *E. coli*) are the major source of urinary tract infections (UTI), sepsis/bacteremia. In addition, ExPEC are the predominant causes of death from bacterial infections among the elderly as well as people with deficient immune response due to malignant diseases, chemotherapy, or immunosuppressive diseases, such as AIDS. They are also an important factor in newborn meningitis (NBM). Since many ExPEC strains are highly resistant to antibiotics and belong to a large number of serotypes obvious treatments, including standard vaccination procedures, are unrealistic. *E. coli* is a heterogeneous species because its genome is highly dynamic and consequently, ExPEC strains associated with human and animal diseases are remarkably diverse. Interestingly, the ability of ExPEC to accumulate and express multiple virulence-associated determinants increases their fitness and adaptability and determines their potential to cause disease. Several such fitness determinants are also shared between ExPEC and commensal strains. These ExPEC virulence factors appear to enable the pathogens to exploit their hosts in ways unavailable to commensal strains, in addition to their role in disease processes, and thus to spread and to persist in the bacterial community.

Our consortium aims at the further characterization of differences between commensal and extraintestinal pathogenic *E. coli* (ExPEC) strains. The scientific objective of this project is to understand the molecular and epidemiological basis for controlling ExPEC infections and to use the knowledge gained for the improvement of diagnostic approaches as well as for the development of new therapeutic and preventive strategies.

The main activities focus on the analysis of the distribution of virulence-associated genes and the structural and functional analysis of selected genomic clusters carrying virulence- and fitness traits. Increased knowledge on the distribution of virulence/fitness-associated genes of ExPEC will further improve proper diagnostics and risk assessment. Furthermore, the consortium works on (i) the functional characterization of selected virulence-associated determinants (i. e., determinants coding for polyketide biosynthesis, sugar utilization, cytolethal distending toxins, fimbrial and other adhesin determinants and iron uptake systems (salmochelin, yersiniabactin) and (ii) the genetic diversity among virulence- and fitness-associated determinants and adaptation of pathogenic and commensal *E. coli*.

Name	**Sebastian Suerbaum**
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Title of the project	HELDIVNET – Parasite and host genetic diversity in Helicobacter infections
Title of your presentation	HELDIVNET – Parasite and host genetic diversity in Helicobacter infections

Helicobacter pylori is the bacterial pathogen with the highest known genetic diversity and variability. The aim of the HELDIVNET ERA-NET consortium, which consists of seven groups led by Mark Achtman (DE, now Ireland), Agnès Labigne and Sébastien Breurec (FR), Marja-Liisa Hänninen (FI), Rainer Haas (DE) Christine Josenhans (DE), José Machado (PT), and Sebastian Suerbaum (DE) was to achieve significant advances in our understanding of *Helicobacter pylori* genetic diversity, the mechanisms that generate it and its functional implications, and the impact of genetic variation on the outcome of *H. pylori*-associated disease. In addition, we aimed at a better understanding of the role of human genetic variation in determining susceptibility to infection with particular *H. pylori* types, and have used comparative genomics and postgenomic functional analysis to better understand the evolution of gastric and enterohepatic pathogenicity within the genus *Helicobacter*.
Important progress was achieved in all areas covered by HELDIVNET and has already resulted in multiple publications, including one published in Science (Moodley, Y. *et al*., Science 2009). This talk will present an overview of the results achieved in the HELDIVNET consortium, which will be complemented by presentations by individual project partners.

Name	**Rainer Haas**
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Title of the project	Genetic characterization of *Helicobacter pylori*-induced gastric carcinogenesis in Mongolian gerbils
Title of your presentation	Helicobacter pylori Type IV secretion systems and pathogenesis in the animal model

Helicobacter pylori colonizes the gastric mucosa of half of the world population and may cause chronic gastritis and peptic ulceration. Disease induction is strongly dependent on the type of *H. pylori* strain. Thus, type I *H. pylori* strains, which carry a functional *cag*-type IV secretion system (*cag*-T4SS) and produce an active vacuolating cytotoxin (VacA) are often associated with more severe disease as compared to type II strains, which lack these virulence factors. The *cag*-T4SS is encoded on the *cag*-pathogenicity island (*cag*-PAI) and consists of at least 27 genes encoding structural proteins involved in forming a bacterial protein injection system. The *cag*-T4SS translocates the effector protein CagA into gastric epithelial cells. Translocated CagA is tyrosine-phosphorylated by the cellular Src kinase. Phosphorylated CagA induces a reprogramming of the cell by affecting the tight junction or adherence junction complex and by inducing cellular motility (cell scattering) and cellular elongation (hummingbird phenotype). The Mongolian Gerbil model is an excellent model to mimick these diseases induced by H. pylori, including gastritis, gastric ulceration and even adenocarcinoma.

In the course of the ERANET programme we finished and annotated the *H. pylori* P12 genome and sequenced the complete genome of the Mongolian gerbil-adapted *H. pylori* strain B128 (renamed after Mongolian Gerbil passage into HPB8). A combined 454-pyro- and Sanger sequencing approach was found to be a very successful method to obtain complete closed genomes. The obtained reads for the HPB8 were assembled in the sequencing centre at the University of Goettingen and annotated at the Bioinformatics Institute, University of Hamburg. It consists of a circular chromosome of 1,673,997 bp and a small plasmid of 6032 bp carrying nine genes. The chromosome contains 1711 coding sequences, 293 of which are strain-specific, coding mainly for hypothetical proteins, and a large plasticity zone containing a putative type-IV-secretion system and coding sequences with unknown function. The *cag*-pathogenicity island is rearranged such that the *cag*A-gene is located 13,730 bp downstream of the inverted gene cluster *cag*B-*cag*1. (Farnbacher *et al*., manuscript submitted).

In a long-term infection experiment (2, 4, 8, 16, 32, and 64 weeks) we challenged Mongolian gerbils with *H. pylori* B8 (WT), or its isogenic mutant that produces CagA, but is unable to translocate it into gastric cells (B8□*cag*Y). By comparing control-, WT-, and mutant-infected animal groups, an early CagA-mediated reprogramming (4 – 8 weeks) of gastric cells occurred only in WT-infected animals. This resulted in a strong cellular proliferation and stomach enlargement, a severe active and chronic gastritis with a significant increase of pro-inflammatory cytokines, mucous gland metaplasia, and atrophy of parietal cells. These early *cag*-PAI-dependent changes were found to trigger later physiological (hypochlorhydria, hypergastrinemia), and histopathological changes, such as gastric ulcer, gastritis cystica profunda, and precancerous lesions (Wiedemann *et al.,* PLoS ONE 4:e4754 (2009)).

Name	**Christine Josenhans**
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Title of the project	Parasite and host genetic diversity in Helicobacter infections
Title of your presentation	*Helicobacter pylori* global diversity and implications for host interaction

Helicobacter pylori is the most variable bacterium characterized to date. Strains isolated from each human host are unique. The variability arises from a high mutation rate and a high propensity to recombine its genome with other coinfecting *H. pylori* strains. The human gastric pathogen has probably coevolved to adapt to individual hosts, who also have a unique genetic and immunological fingerprint (Suerbaum, S. & C. Josenhans, NRM 2007). While housekeeping gene diversity is well investigated and analyzed by MLSA, the global diversity of virulence associated genes is less investigated. DNA sequence analysis of the complete *cag* pathogenicity islands [*cag*PAI, 32 kb; Fischer. W. et al., Mol. Microbiol. 2001] of a representative panel of 29 different ethnic *H. pylori* isolates (Falush, D. et al., Science 2003; Linz, B. et al., Nature 2007; Moodley et al., Science 2009) has been performed and was compared with 9 strains whose whole genome sequences are publicly available. This genetic island not present in all strains encodes a type IV secretion system responsible for the translocation of the virulence factor CagA, and is involved in disease severity. The sequences revealed the structural concordance of the PAIs between strains, except for one subpopulation with a deletion of a set
of genes between HP0536 and HP0546. We have investigated *cag* gene expression and host interaction *in vitro* of these diverse *H. pylori* strains. To evaluate the different potential of strains to induce a host response, we performed infections of human epithelial cells. Infection with *cag*PAI-positive strains leads to increased cell activation, determined by IL-8 secretion. In most strains investigated, IL-8 induction and effector protein CagA translocation were concordant with genetic data from *cag*PAI sequencing, but the amount of IL-8 induced by *cag*PAI-positive strains differed, indicating strain-specific determinants influencing host interaction. Differences in IL-8 induction did not correlate to cell adhesion. Differences in host interaction partially seemed to correlate with gene expression.
We are currently analyzing specific *cag*PAI-encoded proteins with a high rate of non-synonymous mutations, indicating selective diversifying pressure for these changes. The global diversity of
this complex virulence module of *H. pylori* and some functional implications of its diversity will be discussed.

References:

Suerbaum S, Josenhans C (2007) *Helicobacter pylori* evolution and phenotypic diversification in a changing host. Nat Rev Microbiol 5: 441-452.

Fischer W, Puls J, Buhrdorf R, Gebert B, Odenbreit S, Haas R (2001) Systematic mutagenesis of the *Helicobacter pylori cag* pathogenicity island: essential genes for CagA translocation in host cells and induction of interleukin-8. Mol Microbiol 42: 1337-1348.

Falush D, Wirth T, Linz B, Pritchard JK, Stephens M, Kidd M, Blaser MJ, Graham DY, Vacher S, Perez-Perez GI, Yamaoka Y, Megraud F, Otto K, Reichard U, Katzowitsch E, Wang X, Achtman M, Suerbaum S (2003) Traces of human migrations in *Helicobacter pylori* populations. Science 299: 1582-1585.

Linz B, Balloux F, Moodley Y, Hua L, Manica A, Roumagnac P, Falush D, Stamer C, Prugnolle F, van der Merwe SW, Yamaoka Y, Graham DY, Perez-Trallero E, Wadstrom T, Suerbaum S, Achtman M (2007) An African origin for the intimate association between humans and *Helicobacter pylori*. Nature 445: 915-918.

Moodley Y, Linz B, Yamaoka Y, Windsor HM, Breurec S, Wu JY, Maady A, Bernhoft S, Thiberge JM, Phuanukoonnon S, Jobb G, Siba P, Graham DY, Marshall BJ, Achtman M (2009) The peopling of the Pacific from a bacterial perspective. Science 323: 527-530.

Name	**Jesus Pla**
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Title of the project	Glycoshield: surface modulation of the host and fungal response using a genomic approach
Title of your presentation	Glycoshield: surface modulation of the host and fungal response using a genomic approach

The availability of the genomes of the main fungal species with clinical interest provides an exciting opportunity to achieve *traditional* scientific goals with new methodological approaches. The genomes of clinically relevant fungi like *Candida albicans* or *Cryptococcus neoformans* are now available to the scientific community. This is especially relevant as fungal infections represent today a serious and not-yet-solved health problem even in industrialized countries. Infections by both fungal pathogens represent a significant health problem, an economical burden for society and a challenge for research. In both organisms, genetic advances have enabled the identification of determinants of pathogenicity using different test models of infection. In *C. neoformans*, the capsule, a cell surface polymer, has emerged as the major determinant of virulence in this pathogen. In contrast, several genetic traits have been identified in *C. albicans*; in this organism, the host response plays an essential role in virulence. *C. albicans* can therefore be considered an excellent model to understand factors influencing the transition from commensalism to virulence.

In this project a consortium of 7 groups (2 from Spain, 3 from France and 2 from Germany) has focused on the analysis of the role of cellular surfaces as a target to control these infections. Our overall common scientific *leit motiv* subject is glycosylation, as a key mechanism controlling fungal surface properties. We have focused on different steps of the biogenesis of the cell wall in the models indicated above. We have paid attention to the role of different surface components (mannan, glucan and cell wall proteins) and how the cell surface responds to environmental conditions via signalling pathways. We have characterized different SAMs (Surface Altered Mutants) constructed as knock-out strains altered in specific biosynthetic genes. They include (among others) defects in glycosylation (*pmt* and *bmt* proteins among others), specific cell wall proteins (GPI-anchored and non-conventionally secreted proteins (*TSA1*)) and signalling events (MAP kinase pathway-related); we have characterized them in this project using different experimental conditions. We have quantified the exposure of major polymers in the cell surface by flow cytometry, the interaction with host cells, and determined their ability to translocate across epithelial layers. We have also characterized in detail the Interaction among certain components of the O-glycosylation machinery (by the Split Ubiquitin system) to confirm some predicted interactions, identify novel partners of *pmt* proteins as well as identified a novel O-glycosylation protein↔N-glycosylation protein interaction. We have also addressed the role of mRNA splicing in capsule biogenesis in *Cryptococcus neoformans*. From a methodological point of view, we have developed a novel genetic method to screen for non-conventional secreted cell wall proteins, developed a novel method to analyse the cell wall (HR-MAS) and set up an *in vitro* gene reporter system to quantify the interaction of fungal cells and surface components with the innate immune system (TLR2, TLR4 and Dectin-1). *In vivo* studies have characterized the transcriptional response of the fungus during infection at the liver and characterized the genome wide transcriptional response of mouse macrophages against hyphal and yeast forms of the fungus, identifying a core and a morphotype-specific response. A colonization method for *Candida* has been set up in mice and the effects of drugs promoting inflammation and their influence in *Candida* colonization determined. Finally, the role of certain SAM mutants in preventing fungal disease is currently being analysed using an animal model.

Name	**Axel Hartke**
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Title of the project	**Large scale screening of potential key factors involved in the commensalism / virulence transition of *Enterococcus faecalis***
Title of your presentation	idem

Pascale SERROR, Fatima LOPES, Bruno GONZALES-ZORN, Jan KOK, Rob WILLAMS, Johannes HUEBNER and Axel HARTKE

Enterococci are major gut commensals of humans and many animals. Generally considered as harmless, in the last 20 years especially two species (*E. faecalis* and *E. faecium)* have emerged as an increasingly important cause of nosocomial infections with high mortality rates. The most frequent infections are urinary tract infections, intra-abdominal and intra-pelvic abscesses, post-surgical wound infections, subacute bacterial endocarditis, and bloodstream infections. The emergence of resistance against virtually all clinically available antibiotics and the ability to transfer these resistances to other pathogens demonstrate an urgent need for an improved understanding of the fine line that separates a friendly commensal from a frightful opportunistic pathogen.

Methods

E. faecalis V583, a strain isolated from a patient suffering from a persistant bloodstream infection, was selected for the study because it was the first vancomycin-resistant clinical isolate and its annotated genome sequence was available. This strain is part of the high risk clonal complex 2 which comprises mostly isolates derived from hospital infections world wide. A library of targeted insertion mutations in 181 genes has been constructed. Individual mutants were subsequently tested in a first screening for their i) resistance to oxidative stress, ii) antibiotic resistance iii) virulence ability in a surrogate insect model, iv) resistance to opsonophagocytose and v) adherence to the human colon carcinoma Caco-2 epithelial cell-line. Several of the mutants have also been tested in a mouse colonization model. Stable deletion mutants and complemented mutant strains are currently constructed. These will then be tested in a second screening for their i) ability to survive inside macrophages and virulence in a ii) rat endocarditis and iii) different mouse infection models.

From the 181 mutant strains tested in the first screen, 35% showed no detectable phenotype and 48%, 12% and 5 % were tested positive for one, two and more than two phenotypes, respectively. Some of the mutants attracted our special interest. For example, several mutants affected in genes encoding cell surface exposed proteins including a chitin-binding protein, a leucin-rich repeat protein, a cholin-binding protein and putative efflux pumps displayed a phenotype in virulence for *Galleria mellonella*. Interestingly, some mutants had also increased virulence in the insect model. They encode a glycosyl hydrolase and a putative transcriptional regulator homologous to a *Listeria monocytogenes* specific gene. A total of 16 mutations affected phagocytosis and complement susceptibility. Most of these mutants were efficiently killed by a combination of PMNs with complement, while the additional effect of antibody opsonization was negligible. An additional mutant presumably has lost complement resistance through a mutation leading to a defective anchoring of LTA. Two mutants in two adjacent transcriptional regulators displayed significant higher resistance to opsonic killing. The corresponding genes are part of the pathogenicity island of strain V583 and both encode regulators of the same family (TetR). The corresponding mutants are also less virulent in the insect model *G. mellonella*. Other mutants showed significant differences in antibiotic resistance. The most spectacularly change was evidenced for a mutant with an inactivation of the *npr* gene encoding NADH peroxidase. In comparison to the wild-type which was resistant to ceftriaxone, the *npr* mutant was hypersensitive.

The present study contributes significantly to the understanding of *E. faecalis* virulence and confirms the relevance of certain bacterial genes in the pathogenicity of enterococci. Our combined results may lead to the idenfitication of additional targets for alternative treatment and prevention options against otherwise untreatable enterococcal infections.

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Title of the project	**Genomic Approaches to Unravel the Molecular Mechanisms of Pathogenicity in the Human Fungal Pathogen *Candida glabrata***
Title of presentation	As above

Candida glabrata (*C.g*) is an opportunistic human fungal pathogen inherently refractory to antifungal therapy. Like *Candida albicans*, it can cause life-threatening systemic infections in immunocompromised individuals. However, molecular basis of *C.g* virulence and antifungal resistance is not well understood. Based on the *C.g* genome sequence, the FunPath Consortium therefore initiated a large-scale reverse genetic approach to identify novel virulence and drug resistance genes in this non-filamenting fungal pathogen. Based on bioinformatics analysis, we have initially deleted those *C.g* genes having functional orthologues in the non-pathogenic and related yeast *Saccharomyces cerevisiae*, in particular cell wall genes, membrane proteins, signaling pathways and transcriptional regulators. Deletion strains were analyzed for obvious growth phenotypes on a variety of media containing different compounds and drugs, as well as morphology and drug sensitivity and stress response. This approach led to the identification of *C.g* genes implicated in metal ion or detergent tolerance, as well as novel genes implicated in resistance to cell wall-perturbing compounds and antifungal drugs.

In our presentation of the FunPath consortium, we will report on the progress of the global profiling experiments, and we will report about technology developments such as bar-code chips, which were developed in the consortium. Furthermore, we will report about the functional characterization of selected deletion strains with interesting phenotypes related to virulence or drug resistance. For instance, lack of the *C. glabrata CBK1* gene causes a severe growth defect, as Cg*cbk1*Δ cells are unable to separate properly, form large cell aggregates with very large, rounded cells displaying severe drug hypersensitivity. Notably, absence of the transcription factor Ace2, which also causes a hypervirulence phenotype, leads to a similar growth and cell aggregation phenotype. Interestingly, Cg*cbk1*Δ and Cg*ace2*Δ cells are not phagocytosed by murine bone marrow-derived macrophages (BMDM) *in vitro*. The inability to internalize the large cell aggregates formed by these mutants is reversed when cell aggregates are separated into single cells prior to infection or by complementation with the endogenous gene. We propose that the inability to phagocytose large cells aggregates and the differences in the cell wall composition may changes in immune response of the macrophages or perhaps the host, leading to hypervirulence of Cg*ace2*Δ cells. Interestingly, transcriptional profiling identified not only genes regulated by CgCbk1 via the transcription factor CgAce2, but also differential expression of a novel target, *EPA13*, a member of the large family of epithelial adhesins (*EPA*). Moreover, CgCbk1 also controls several amino acid biosynthesis and metabolic genes, as well as cell wall biogenesis genes, suggesting extensive transcriptional rewiring of the *C.g* Cbk1 network when compared to the orthologue Cbk1 from bakers yeast.

Finally, after the start of FunPath, we have initiated a collaboration with the groups of Ken Haynes and Brendan Cormack, who have generated a library of more than 200 viable *C.g* transcription factor mutants, all of which were combined with the FunPath collection. All in all, the *C.g* deletion collection now comprises more than 700 verified deletion strains, all of which have been subjected to phenotypic profiling. This collection of *C.g* deletion mutants is now one of the largest mutant collections of a fungal pathogen and thus will serve as a useful toolbox for the scientific community studying *Candida glabrata* biology and pathology *in vitro* and *in vivo*.

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Title of the project	European Initiative to Fight Chlamydial Infections by Unbiased Genomics - ECIBUG
Title of your presentation	European Initiative to Fight Chlamydial Infections by Unbiased Genomics - ECIBUG

ECIBUG continued its work with fully established cooperations. Based on the infrastructure set up previously with success, the consortium shared novel genomic data and materials to achieve the goal of ECIBUG: to understand the pathobiology of chlamydial infection on a genomic level. Current main achievements of our pan-genomic approach to analyze intracellular survival of *C. pneumoniae* and its diversity are:

- As a result from two complementary high-throughput screening (HTS) approaches about 70% of *C. pneumoniae* effector proteins with type III secretion signals were identified incl. 15 proteins whose expression in yeast also affects yeast growth and that are now studied further according to a priority list.
- siRNA loss of function HTS for *C. trachomatis* using kinase libraries revealed 59 hits that alter infectivity.
- In-depth analysis of postinfectiously modified human signaling cascades *in vitro* and in part *ex vivo* focused on Endothelin-1 and WNT signalling, p53, ERK/MEK, RAF1/AKT1, SNARE proteins and hsp60 related stress associated molecules.
- Establishment of novel polarized epithelial cell model for chronic chlamydial infection.
- yeast-two hybrid screening performed with four *C. pneumoniae* effector proteins revealed tubulin and other potential interacting partners.
- Definition of 7 novel synthetic and 9 novel natural antichlamydial compounds.
- Novel impedance based bioassay to follow chlamydia infection using the non-invasive Electric Cell-substrate Impedance Sensing-technique (ECIS) based on microelectrodes integrated into surfaces compatible with cell growth.
- Identification and characterization of three new chlamydial cell surface proteins with attachment properties.
- 10 candidate substrates for anti-apoptotic chlamydial protease CPAF identified via proteomics screen.
- Identification of a direct CPAF inhibiting agent that inhibits chlamydial development.
- Ongoing sequencing studies of several chlamydial isolates to identify genetic clusters

ECIBUG has created an environment permitting its partners to share data without restraint and to focus complementary approaches onto the common objective of understanding and eradicating chlamydial infection. Taken together, ECIBUG is progressing as planned and there was no need to amend the general objectives of the project. Several projects will be cost-neutrally extended beyond January 2010 to provide comprehensive analysis of the data obtained.

Name	**Agathe Subtil**
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Title of the project	ECIBUG/PATHOMICS
Title of your presentation	Effector proteins during *Chlamydia* infection: identification and roles

Within the ECIBUG project, two independent approaches were conducted to identify novel proteins secreted in the host cell during infection by the intracellular pathogen *Chlamydia*. This collaborative work succeeded in identifying about 15 novel potential effector proteins, all of which are specific to chlamydiae. About half of them are probably Inc proteins, translocated on the membrane of the compartment in which the bacteria develop, the inclusion. We will present some data that our group obtained regarding the function of one effector protein of chlamydiae. We showed that this protein was implicated in the import of lipid droplets and peroxisomes within the inclusion. The discovery of the presence of peroxisomes in the inclusion prompted us to undertake the identification of some of the lipids produced during infection. This last point is currently being studied within the frame of the PATHOMICS consortium, in the Pathogenomics 2nd call.

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Title of the project	ECIBUG
Title of your presentation	**European initiative to fight Chlamydial infections by unbiased genomics**

Chlamydia pneumoniae is a common respiratory pathogen. The epithelium of the respiratory tract constitutes a physical barrier and the first line of host defence that *C. pneumoniae* encounters. Epithelial cells lining the respiratory tract are architecturally structured in a polarized orientation with distinct apical and basal faces.

Various cell culture models have extensively been used to study characteristics and pathogenesis of chlamydial infections but most published papers with epithelial cell lines report infection in cell cultures grown on impermeable plastic or glass supports, in which case the membrane asymmetry of a polarized cell and proper cell-cell contacts forming the tight barrier across the cell layer are lacking.

A polarized cell culture model was set up to study the pathogenicity and cytokine responses of *C. pneumoniae* infection. The ability of two cell lines, human Type II pulmonary epithelial cell line A-549 and lung adenocarcinoma cell line Calu-3, to form polarized cultures was studied in more detail. Immunofluorescence staining patterns of a tight junction protein and measurements of transepithelial resistance indicated proper polarization and formation of functional tight junctions in Calu-3 cells but not in A549 cells grown on semipermeable supports.

The primary infection in polarized Calu-3 cultures produced more *C. pneumoniae* (measured as genome equivalents) than infection in the flat cultures. The progeny from polarized Calu-3 cultures, however, was not as infective as that released from flat cultures. The infection in polarized Calu-3 cells was quite resistant to the inhibitory effects of doxycycline: a concentration of 2 µg/ml in the primary culture could not prevent growth of *C. pneumoniae* after repassaging. Also, several cytokines were released mainly on the apical side of the polarized Calu-3 cells in response to *C. pneumoniae* infection. Preliminary studies with a plant phenolic compound luteolin (shown to be effective against *C. pneumoniae* in flat HL cultures) in polarized Calu-3 cells showed some inhibition of chlamydial growth at a concentration of 63 µM, but this concentration was harmful to the cells as well.

These findings indicate altered growth of *C. pneumoniae* in filter-grown cells cultures compared to the conventional flat monolayer cultures. Low production of infective progeny of *C. pneumoniae* together with resistance to antibiotics and polarized secretion of cytokines from infected Calu-3 cells could indicate that this model might better resemble the *in vivo* epithelial cell barrier than the conventional cell culture models.

Also topics and results from partner 4 (Prof. Pia Vuorela) will be shortly introduced in this presentation.

Name	**Jean-marc Ghigo**
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Title of the project	Exploring Protein Secretion within the bacterial biofilm matrix. Acronym: EPS-Matrix
Title of your presentation	Exploring Protein Secretion within the bacterial biofilm matrix.

The development of surface-attached biofilm bacterial communities is commonly associated with health and economic problems when colonizing industrial or medical surfaces. One of the most characteristic biological features that distinguish biofilms from planktonic populations is the production of an extracellular matrix embedding the biofilm bacteria. The objective of the *EPS-Matrix* project was to identify matrix proteins and other molecules that could play new biological roles in biofilms formed by *Escherichia coli, Salmonella enterica* serovar Enteridiidis, *Pseudomonas aeruginosa* and *Staphylococcus aureus.* Soluble molecules potentially accumulating in the matrix of mature biofilms formed in microfermentors were extracted and analyzed using different approaches including proteomic analysis as well as different phenotypic assays. In parallel, *in silico* genomic analysis of potential bacterial secretion systems potentially delivering proteins within the matrix was used to investigate protein-mediated biofilm development. The contribution of the identified secreted molecules, as well as their biofilm specificity was carried out by 5 groups (3 in France, 2 in Spain) with complementary expertise. The scientific activity directly derived from this program led, in particular to the study of *S. aureus* proteinaceous-dependent biofilm matrix and the demonstration of the role of LPXTG protein and Fibronectin binding proteins in biofilm formation. It also led to the functional characterization of a family of 7 chaperone-usher fimbriae previously considered as cryptic. The methodology of biofilm-matrix extraction used in the program also led to the identification of polysaccharidic polymers produced within the *E. coli* and *P. aeruginosa* biofilm matrix. The program is still in progress regarding functional analysis of the type 2 and type 6 secretion pathways in *E. coli* and *P. aeruginosa*. The biological relevance of the findings directly derived from the program and their contribution to the understanding of the biological role performed by the bacterial biofilm matrix will be discussed.

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Title of the project	*Pneumocystis* PathoGenoMics: Unravelling the Colonization-to-Disease Shift
Title of your presentation	*Pneumocystis* PathoGenoMics: Unravelling the Colonization-to-Disease Shift

Most of the available data on *Pneumocystis* basic biology were acquired using *Pneumocystis* organisms developing in deeply immunodepressed experimental or human hosts. However, the understanding of *Pneumocystis* development in healthy hosts appears as a critical step to clarify how *Pneumocystis* organisms, which cannot be continuously cultured, shift to pathogenic proliferation in susceptible or naïve hosts. In other words, which are the biological changes undergone by *Pneumocystis* organisms that allow them to proliferate extensively in the lungs of susceptible hosts and to cause pneumonitis, potential cause of respiratory failure? Our proposal focused on this question.

The present project is a transnational, post-genomic basic-biology joint research program that targeted *Pneumocystis* replication mechanisms, life-cycle stage transitions and relationships of these processes with the pathological changes associated with *Pneumocystis* pneumonia (PcP). This project emerged at a special time for *Pneumocystis* research as new innovative scientific and technical post-genomic approaches become feasible thanks to the nearing achievement of the *P. carinii* genome sequencing. The selected strategy proposed a comprehensive exploitation of efficient experimental models of *Pneumocystis* colonization and infection, associated with the application of transcriptomic, proteomic and other post-genomic approaches. The targeted species were *Pneumocystis carinii* (from rats), *P. murina* (from mice), and *P. jirovecii* (from humans).

The setting up a reliable separation method (FACSAria Cytometer, Becton Dickinson) of *Pneumocystis carinii* life cycle stages was a major result of the work developed in the framework of the present project. Separated *P. carinii* life cycle stages were used to explore the relative contribution of *Pneumocystis* life cycle stages to *Pneumocystis* proliferation, and also at determining whether the trophic-to-cystic form transition is required for life cycle completion. Growth kinetics of either pure trophic or cystic forms of *P. carinii* was followed in vitro and in vivo. On the whole, trophic forms can apparently multiply in vitro on their own but they cannot develop into cystic forms, in contrast to what happens in the rat model. This observation may explain the absence of continuous growth of *Pneumocystis* in vitro. Furthermore, DNA intercalating agent (Sytox® green dye, Invitrogen) allowed us to measure DNA contents of sorted trophic and cystic forms (in comparison with haploid and diploid yeast reference strains) providing important data to the understanding of *Pneumocystis* life cycle. Regarding *P. murina*, only some genes of this species – all involved in cell division (*Cdc25, C1-THFS, GSC-1*), have been found differentially expressed in the shift from colonization to PcP, but these results need still to be confirmed.

Regarding *P. jirovecii*, different proteomic profiles were found in bronchoalveolar fluid (BALF) samples from Idiopathic Pulmonary Fibrosis (IPF) patients with or without *Pneumocystis* colonization. Furthermore, in chronic obstructive pulmonary disease (COPD) patients, serum concentrations of IL-8, TNF, IL-6, and MCP-1 were all significantly elevated in the colonized patients compared with the noncolonized controls. Since high levels of airway and systemic inflammatory markers are associated with a faster decline in lung function, the presence of *P. jirovecii* in these patients could contribute to COPD pathology. Finally, the ability of colonized patients to transmit the infection to susceptible ones was also explored. Thus, we have provided molecular evidence that transmission of *P. jirovecii* from colonized immunocompetent carrier hosts to susceptible persons may occur and that *P. jirovecii* transplacental transmission could occur in humans. These findings opened new fields to *Pneumocystis* research.

Name	**Patrick Trieu-Cuot**
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Title of the project	A comparative molecular analysis of GAS and GBS pathogenesis
Title of your presentation	Role of pili in the virulence of streptococci

Most bacterial pathogens have long filamentous structures known as pili or fimbriae extending from their surface. These hairy structures are often involved in the initial adhesion of bacteria to host tissues but also in bacteria-bacteria interaction resulting in biofilm formation. In Gram-negative bacteria such as uropathogenic *Escherichia coli*, pili are formed by the non-covalent interactions between protein subunits called pilins. In contrast, the recently discovered pili in Gram-positive pathogens are formed by covalent polymerization of pilin subunits. *Streptococcus agalactiae* (Group B Streptococcus) is a leading cause of sepsis (blood infection) and meningitis (infection of the fluid and lining around the brain) in newborns. This bacterium also causes infections in the elderly and in adults with underlying diseases. We characterized the pilus-encoding operon PI-2A (gbs1479-1474) in strain NEM316. This pilus is composed of three structural subunit proteins: PilA (Gbs1478), PilB (Gbs1477), and PilC (Gbs1474), and its assembly involves two class C sortases (SrtC3 and SrtC4). PilB, the *bona fide* pilin, is the major component whereas PilA, the pilus associated adhesin, and PilC are both accessory proteins incorporated into the pilus backbone. The covalent polymerization of the pilus is due to the action of two enzymes belonging to the class C sortase family. We showed that the PilB fiber is essential for efficient PilA display on the surface of the capsulated strain NEM316 and, more importantly, that pilus integrity becomes critical for adherence to respiratory epithelial cells under flow-conditions mimicking *in vivo* situation. The adhesin PilA contains a von Willebrand adhesion domain (VWA) that is essential for its adhesive function. S. agalactiae strain NEM316 was able to form biofilm and, strikingly, the PilA and PilB mutants were strongly impaired in biofilm formation. Surprisingly, the VWA domain involved in adherence to epithelial cells was not required for biofilm formation.

Although pili are described as adhesive organelles, they have been also implicated in many other functions including thwarting the host immune responses. We thus investigated the possible role(s) of PilB in subverting macrophage function(s). Phagocytosis and survival of wild-type NEM316 and its isogenic Δ*pilB* mutant in immortalized J774A.1 and RAW 264.7 macrophages were not significantly different. This result was confirmed using primary peritoneal macrophages. In these assays, macrophage activation was assessed by determining the levels of TNF-α, IL-12, and IL-6, as well as nitrite production. The ability of the Δ*pilB* mutant to activate macrophage responses was significantly reduced. Nitric oxide (NO) being a key player of GBS-induced murine macrophage apoptosis, we tested macrophage apoptosis using annexin-V flow cytometric assays. Apoptosis was reduced with the Δ*pilB* mutant in comparison to the parental strain. The role of PilB was tested in systemic virulence using 6-weeks old and newborn mice. Notably, the non-piliated Δ*pilB* mutant was less virulent than its wild-type counterpart in the newborn mice model. Taken together, these results suggest a role for the streptococcal fiber in fine-tuning the host immune responses that, in turn, can be important for bacterial dissemination *in vivo*.

Name	**Philippe Glaser**
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Title of the project	A comparative molecular analysis of GAS and GBS pathogenesis
Title of your presentation	Expression of surface proteins in GBS

Genes encoding surface antigens are highly variable among GBS isolates. They are frequently carried by genomic islands and are acquired by lateral gene transfers. However, in the course of commensalism and in the different phases of the diseases in human and animal hosts, their expression should be tightly regulated in a coordinated manner. These regulations involve global regulators carried by the genome backbone and regulators associated with the structural genes encoding these surface proteins both located on genomic islands. We have characterized the regulatory networks controlled by the two-component systems CovRS and DltRS which have a global effect on the expression of surface components and of two pairs of paralogous regulators of the AraC and Rgg family associated with loci encoding pili and membrane bound proteins. This analysis revealed that *Streptococcus agalactiae* has evolved cross talks between regulators as a means to coordinate the expression of genes encoding cell surface proteins.

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Title of the project	Systematic analyses of kinase and phosphatase function in morphological, environmental, and virulence responses of the human fungal pathogen *Candida albicans*
Title of your presentation	Systematic analyses of kinase and phosphatase function in morphological, environmental, and virulence responses of the human fungal pathogen *Candida albicans*

An important factor in the pathogenicity of *Candida albicans* is its ability to exhibit a large morphological variability in response to changing environmental conditions. In particular, the morphogenetic switch between the yeast and filamentous forms is thought to be an important virulence trait. Furthermore the ability of this fungi to switch from a normal yeast form (white) to an elongated, mating competent form (opaque), enables *C. albicans* to adapt efficiently to different host niches. Correct cell cycle regulation is critical for these different growth and developmental stages and the respective transition/switches between them.

In order to systematically identify regulators of the yeast to filamentous transition, white-opaque switching, cell cycle progression and responses to a range of stresses, we expressed all putative protein kinases, phosphatases, and their regulators (123 kinases, 25 kinase regulators, 39 phosphatases, and 6 phosphatase regulators, which were identified in the *C. albicans* genome[1]), as well as >30 predicted hyperactive or dominant-negative alleles of these genes from a tetracycline-inducible promoter (2) in two different strain backgrounds (the clinical isolate SC5314 and the *MTL*a strain WO-1). After screening two independent clones of each strain expressing each kinase, phosphatase or regulator we have identified more than 20 proteins (kinases and phosphatases) whose forced expression affects filamentation and 4 kinases whose forced expression efficiently stimulates white - opaque phase switching. In addition these constitutive expression libraries were used to analyze kinases and phosphatases which play a role in cell cycle progression and a range of stress responses. We will discuss the different screens and proteins identified as well as our molecular analyses of these candidates in the various processes.

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TALKS: 2nd call projects

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Title of the project	Genome wide screening of the human pathogen Neisseria meningitidis for proteins enhancing serum resistance and evaluation of their vaccine potential
Title of your presentation	Exploring adaptation to serum stress by meningococci

The consortium seeks to identify meningococcal proteins enhancing serum resistance, which is a major factor driving meningococcal pathogenicity. Serum resistance is mostly mediated by the capsular polysaccharide. However, the identification by Madico et al. (J. Immunol 2006) of the investigational vaccine antigen GNA1870/LP2086 as the factor H binding protein Fhbp demonstrated that subcapsular structures support serum resistance, e.g. by employing complement regulator proteins. Meningococcal genomes have not been fully mined in this respect. Furthermore, the meningococcus alters global gene expression under changing environmental conditions that might bring into play factors not expressed under standard in vitro conditions.

In the project, we will attempt to select serum resistant variants by testing diverse environmental conditions and by employing a transgenic animal infection model. We will characterize variants by means of transcriptomics and proteomics. The proteins shall be assessed for their vaccine potential in later phases of the project.

After a first joint meeting in May 2009, the staff recruitment phase was finalized in August. The group agreed to use meningococcal B strains from the clonal complexes ST-32 and ST-41/44. 12 strains -derived from asymptomatic carriage and invasive disease - were mutated in genes encoding possibly dominant mediators of serum resistance, i.e. the capsule, LPS sialylation, and the factor H binding protein GNA1870. Mutant strains lacking capsule, LPS sialylation, and Fhbp are currently subjected to various environmental conditions to assess enhancement of serum resistance. Besides the analysis of pathogen derived mutants we also analyse capsule null locus meningococci (Claus et al. Microbiol 2002) which serve as a model for natural adaptation of unencapsulated meningococci to the host.

Sequential passages of meningococcal strains in transgenic mice expressing human transferrin (Zarantonelli et al. Infect Immun 2007) showed that bacteria better survived in vivo after repeated animal passages. The phenotype, however, was lost after growth on agar plates suggesting regulatory rather than mutation events. Methods will be established to retrieve bacteria directly from the blood for consecutive transcriptome analysis.

As host species specificity is a clue to unravel molecular mechanisms of complement defense in pathogenic Neisseria, animal infection experiments will further be extended e.g. by using mice supplemented with (or transgenic for) human factor H. Furthermore, the ability of meningococci to bind complement inhibitors from different animal species will be examined.

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Title of the project	Functional genomics of host-pathogen interactions using high-throughput screenings: a novel approach towards identifying therapeutic/prophylactic targets**.**
Title of your presentation	Functional genomics of host-pathogen interactions using high-throughput screenings: a novel approach towards identifying therapeutic/prophylactic targets**.**

Early views of infectious diseases presumed that the outcome of the disease was mainly dependent on intrinsic properties of pathogens. In contrast, it is currently accepted that neither pathogen nor host-related features can independently characterize the progress of infections. During the infection process, pathogens try to modulate different host pathways for their own benefit. The final outcome depends on the concerted action of a repertoire of virulence factors which, in turn, can be counteracted by host defense systems activated by the same or other virulence factors. Therefore, further development of our current knowledge requires undertaking large scale analysis of the host-pathogen interplay.

This project aims to identify novel pathogen determinants targeting host functions. Our efforts are placed on the study of five important human pathogens: *Mycobacterium tuberculosis*, *Legionella pneumophila*, *Helicobacter pylori*, *Neisseria gonorrhoeae* and *Klebsiella pneumoniae*. Crucial goals of this project are the **development of host cellular assay systems** amenable to automation and the **construction of transposon-based mutant libraries** of the pathogens under study. Both tools will be used in **high-throughput analysis** employing flow cytometry, luminometry and automatic microscopy as functional read-outs, robotic infra-structure and in-house data analysis. The proposed research program is expected to uncover novel virulence determinants from a collection of human bacterial pathogens which may lead to develop innovative therapies based on the modulation of the host-pathogen interface.

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Title of the project	UTI-Interference
Title of your presentation	Characterization of fitness traits and genome plasticity of asymptomatic bacteriuria *E. coli* isolates

Urinary tract infections (UTIs) are among the most common bacterial infectious diseases of humans and a major cause of morbidity and mortality. Asymptomatic bacteriuria (ABU), is probably the most common form of UTI. ABU is an asymptomatic carrier state that resembles commensalism. In contrast to acute pyelonephritis isolates, the clinically benign nature of many ABU isolates results from the frequent inactivation of virulence-associated determinants, consistent with poor adherence and cell contact, which are essential to trigger the innate host response. ABU may be described as an example of bacterial interference. During long periods of time, patients are colonized with the same strain, and if left untreated, superinfection with other strains seems to be hindered. Consequently, ABU protects against recurrent episodes of symptomatic recurrences.

Our understanding of the molecular basis of the ABU phenomenon as well as the clinical effectiveness of ABU isolates to prevent symptomatic UTI is still incomplete and requires further investigation. A thorough analysis and deeper understanding of the molecular mechanisms of bacterial adaptation to living conditions in the host are essential for the understanding of ABU, for the development of therapeutic strategies based on ABU *E. coli* strains to treat recurrent UTI, and the identification of bacterial factors required for symptomatic infection. For this purpose, we establish a representative collection of ABU isolates that is subjected to molecular epidemiology and analysis of the distribution of ExPEC virulence- and fitness-associated genes. To identify traits contributing to the ABU phenomenon, relevant genomic parts of selected ABU *E. coli* strains are functionally analyzed with respect to their role in asymptomatic bladder colonization. In addition to genome plasticity, differences in virulence gene expression seem to be responsible for the virulence potential and for host adaptation. We therefore need a better understanding of the expression of virulence genes and the potential of ABU strains to induce a host response upon bladder colonization. To analyze changes in gene expression levels in response to prolonged growth of *E. coli* 83972 in the urinary tract, we examine the transcriptome and proteome in selected re-isolates of ABU strain 83972 in comparison with the “wild type” strain. The expected achievements of this project are to improve current strategies to combat recurrent UTI by the use of alternatives to antibiotics and the identification of alternative targets for vaccine development and therapeutic approaches.

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Title of the project	ADHRES signature project
Title of your presentation	Expression profiling of adhesive (ADH) and resistance (RES) genes in Pseudomonas species biofilm lifestyle through a transcriptomic approach

Our goal is to propose a core set of genes involved in biofilm development and antimicrobial resistance that are important in *P. aeruginosa*, *P. putida* and *B. cenocepacia* in relation to the *in vivo* infectious situation and that can be relevant to clinical pathogenicity of the strains, list that can be easily implemented and patentable to become a subset of genes that can be screened by chips or any other technique dedicated to mRNA levels evaluation. ADHRES chips containing biofilm and resistance genes are under construction for these microorganisms. The ADHRES Signatures (corresponding to expression of selected genes) will be analyzed in *P. aeruginosa*, *P. putida* and *B. cenocepacia* strains from human infections and compared to referenced ADHRES-Signatures obtained in standardized biofilm growth conditions mimicking *in vivo* infections. Identification of unravelled genes involved in resistance by mutagenesis is coupled to this approach to implement the ADHRES chips.
The presentation will focus under the tasks already achieved at the end of the 1st year.

1. Presentation and implementation of the existing ADH *P. aeruginosa* chips with genes involved in *P. aeruginosa* antimicrobial resistance leading to ADHRES *P. aeruginosa* chips
2. Design of the *P. putida* and *B. cenocepacia* ADHRES chips with coupled approaches including bioinformatics, *in silico* analysis and *in vivo* screenings as well as quantitative proteomics or transcriptomics analysis.
3. Identification of unravelled genes involved in antimicrobial resistance by mutagenesis in *P.aeruginosa, P. putida and B. cenocepacia* mutagenesis in *P. putida* reference clinical strains and development of new molecular tools that could circumvent the multidrug resistance problem of clinical strains by the choice of an appropriate counterselection marker.

Furthermore, to facilitate the collaborative work between the partners of the project, a WIKI has been set up which is a secured publication system where each partner can exchange their data, comments and so on.
Thanks to participation of the project in the acquisition of a SOLID sequencer, Partners have decided to sequence new strains of *P.aeruginosa, P. putida and B. cenocepacia*

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Title of the project	*sncRNAomics* - High throughput comparative sncRNAome analysis in major Gram-positive human pathogenic bacteria: functional characterisation by a systems biology approach and peptide nucleic acid drug design
Title of your presentation	Current status of sncRNA analysis of *Listeria monocytogenes*, *Staphylococcus aureus, Streptococcus pyogenes, Enterococcus faecalis* and *Clostridium difficile*

In the recent years, the number of drug- and multi-drug-resistant microbial strains has increased rapidly. Therefore, the need to identify innovative approaches for development of novel anti-infectives and new therapeutic targets is of high priority in global health care. The detection of small non-coding RNAs (sncRNAs) in bacteria has attracted considerable attention as an emerging class of new gene expression regulators. In many respects, sncRNA screens in Gram-negative bacteria have set a blueprint for the global and functional identification of sncRNAs for Gram-positive microbes, but the functional role of sncRNAs in colonisation and pathogenicity for *Listeria monocytogenes*, *Staphylococcus aureus, Streptococcus pyogenes, Enterococcus faecalis* and *Clostridium difficile* is almost completely unknown. Here, we report the current status about the sncRNAs analysis of these five Gram-positive pathogens, and compare high-throughput microarray data of our sncRNA screens with computational sncRNA predictions for genome wide sncRNA identification. Finally, we show some preliminary results of the application of modified peptide nucleic acids (PNAs).

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Title of the project	TRANSPAT
Title of your presentation	Transcriptional networks controlling virulence in filamentous fungal pathogens (TRANSPAT)

Despite the advances in preventive, diagnostic and therapeutic interventions, the incidence of invasive fungal infections in immunocompromised patients continues to increase. A significant number of invasive mycoses are now associated with filamentous species (moulds). The available therapeutic options against filamentous mycoses are limited, because they fail to respond to existing antifungal agents, leading to the highest mortality rates for any bloodstream infections.

A critical step in systemic fungal infection is dissemination of the microorganism via the bloodstream. How filamentous fungi are able to survive in the hostile environment of mammalian blood to invade the underlying tissues remains largely an open question. Even less is known about the mechanisms of clinical resistance, i.e. the ability of these pathogens to cause fatal infections despite the administration of antifungal agents showing in vitro activity.

The starting hypothesis of the TRANSPAT project is that invasive fungal infection critically depends on transcriptional networks controlling sets of target genes required for survival and dissemination in the bloodstream. This hypothesis is based on the finding that knockout mutants that lack key transcription factors are strongly attenuated in virulence. The work of the participating teams will focus on two filamentous pathogens, *Aspergillus fumigatus* and *Fusarium oxysporum*, both causing systemic infections in humans with mortality rates as high as 85-90%

TRANSPAT exploits the availability of complete genome sequences and existing collections of key virulence mutants in *A. fumigatus* and *F. oxysporum*. Oligonucleotide microarrays are used to compare expression profiles between wild type strains and knockout mutants lacking key transcription factors. Experiments are performed in an *ex vivo* model mimicking bloodstream infections. This approach will identify gene sets showing altered expression during invasive infection and/or exposure to antifungals. As a parallel and complementary appoach, digital gene expression (DGE) analysis through Massively Parallel Signature Sequencing will be performed, allowing quantitative measurement of individual RNA molecules in the entire transcriptome.

In order to identify direct transcriptional targets, chromatin immunoprecipitation (ChIP) will be carried out under blood growth conditions, using fungal strains expressing tagged versions of key transcription factors. The combination of ChIP with massive parallel sequencing (ChIP-Seq) will allow to globally locate genomic sites bound by these factors *in vivo.* Data from microarray, digital gene expression and ChIP-seq analysis will be integrated to detect significant changes in gene expression and to model regulatory transcriptional networks.

In a final step, functional analysis of candidate target genes will be carried out in order to validate their clinical relevance. Expression levels of selected fungal target genes will be measured in infected mice, using real time qPCR. Selected candidate target genes will be knocked out in *Aspergillus* and *Fusarium,* allowing phenotypic characterization and virulence assessment in murine models of systemic infection.

The primary goal of TRANSPAT is to create a comprehensive picture of the regulatory networks controlling invasive fungal infection. The obtained insights will expand our current knowledge on how filamentous fungi use their transcriptional repertoire to overcome host responses and the deployment of antifungal drugs, and should have direct applications for improving the clinical outcome of invasive fungal infection.

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Title of the project	The cell wall as a target to improve antifungal therapy against Aspergillosis
Title of your presentation	Signal transduction and biosynthesis of the cell wall of *Aspergillus fumigatus*

Aspergillus fumigatus is the most important airborne human-pathogenic fungus. *A. fumigatus* has the potential to cause life-threatening diseases in immunocompromised patients called invasive aspergillosis (IA). In the last two decades, the incidence of IA has tremendously increased since therapeutic intervention is limited. The mortality of IA is very high ranging between 50-90 % despite therapy. The cell wall of *A. fumigatus* is a unique structure, which does not exist for human cells. It enables the fungus to resist against external aggressions, but, at the same time, it is its Achilles' heel since it is a major drug target as shown by the commercial launch of echinocandins that block cell wall biosynthesis. Polysaccharides represent the major part of the fungal cell wall and are responsible for its rigidity and plasticity. Five structural polysaccharides are present in the cell wall of *A. fumigatus* mycelium and conidia: ß-1-3) glucan, chitin, galactomannan, □-(1-3)-glucan and ß-(1-3/1-4)-glucan. ß-(1-3)-glucan is highly branched with ß-(1-6) linkages constituting the core of a three-dimensional network with a large number of side-chains and ramifications. Other polysaccharides such as chitin, galactomannan and ß-(1-3/1-4) glucan are cross-linked to the branched ß-(1-3/1-6) glucan network. Besides polysaccharides, the *A. fumigatus* cell wall contains many secreted proteins. However, no proteins covalently bound to cell wall polysaccharides have been found in *A. fumigatus*. The exact structure of the cell wall and in particular its biosynthesis have not been understood until today. Perturbations of the cell wall e.g. by the use of cell wall-disturbing drugs trigger a repair mechanism that reconfigures its molecular structure to preserve the cell integrity. Cell wall perturbations trigger a regulatory response of the fungus which can, at least in part, overcome the toxicity of the drugs by enhanced biosynthesis of polysaccharides and/or by activating salvage pathways. This is an increasing problem in clinical settings. To understand these regulatory responses and to identify new targets for drugs or combinations of drugs which could be used to prevent the effectiveness of such salvage pathways, we are aiming at dissecting the chemical structure of the cell wall and the regulatory and signal transduction mechanisms controling the cell wall biosynthesis of *A. fumigatus*. The interplay of the different signal transduction pathways and cell wall biosynthesis is extremely complex. Until now, we have identified several members of signal transduction (e.g., the MAP kinase MpkA) and transcription factors controlling the cell wall biosynthesis. Also, potential salvage pathways like the formation of pyomelanin have been identified. Methods of functional genomics like proteome and transcriptome analysis have been extensively used to identify potential pathways regulating cell wall biosyntheses and restructuring of the cell wall during perturbations. I will summarize the signal transduction pathways linked to cell wall biosynthesis and talk about novel aspects on potential salvage pathways and regulatory networks controlling cell wall biosynthesis.

Our EraNet project ANTIFUN combines the groups of Jean-Paul Latgé (Institut Pasteur, France), Emilia Mellado (Instituto de Salud Carlos III, Spain), Olga Genilloud (Fundación Centro de Excelencia MEDINA, Spain) and Axel Brakhage, Olaf Kniemeyer (HKI, Germany)

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Title of the project	**Pathogenomic of increased *Clostridium difficile* virulence**
Title of your presentation	As above

Clostridium difficile-associated infection (CDI) is currently the most frequently occurring nosocomial infection in many European hospitals. *C. difficil*e induces severe diarrhoea in patients with compromised normal gut flora mostly due to antibiotic therapy. The increased incidence and severity of CDI is in part associated with some genotypes, e.g. ribotypes 027, 078, 017, 053.

The aims of this project are to define *C. difficile* groups with increased virulence potential (hypervirulent strains) and analyze their genomic characteristics in terms of the presence of known and new virulence factors, regulation of their expression and genomic heterogeneity.
It is known that there is a considerable degree of genome variation between different strains of *Clostridium difficile*, stemming from mutations, recombination, horizontal gene transfer and from mobile genetic elements which are found at high frequency in the sequenced genomes.
In order to identify sequence variations that can be correlated with virulence properties a collection of (hyper)virulent strains was composed by consortium members. Within this project hypervirulence or increased virulence potential was defined as property to spread and prevail in a given time and region and/or to cause fulminate form of the disease. Strain collection including several epidemic strains from documented outbreaks infecting both animal and human hosts was distributed to all partners.
In the initial phase strains were typed by two ribotyping approaches and by MLST. This grouping will enable selection of related strains with low virulence potential that will be used as control strains. Strain collection was used also for phenotypic characterization including toxin production, colony variability, sporulation efficiency studies as well as for construction of knockout mutants. Sporulation studies included analysis of *skin*-like element that must be inserted into the gene for σ^K for efficient sporulation in *C. difficile*. PCR based assay is under development to determine whether any variation in the efficiency of sporulation could be at least in part explained by the presence/absence or variability of a *skin*-type element.
Analysis of intergenic regions (ITS) in ribosomal operon was performed in several ribotypes. In *C. difficile* genome the rRNA operon is present in several copies that differ in the length of ITS and a single primer pair can result in a pattern of bands ranging from 200 - 700 bp. ITS regions were amplified and individual bands were purified from gel, cloned and sequenced. Characterization of ITS sequences demonstrated mosaic-like structure with sequence blocks that are present or absent in different rDNA copies. However, no type specific ITS sequences that could be used as molecular markers were found.
For genomic analysis we are now in the process of implementing a rational database that can store data as genbank and fasta files and as output of different gene finding software, and that allows fast access to the data. Also we are in the process of evaluating different gene finding software programs for their use in the project.
Alternative animal models were included in the project with aim to develop assay for increased virulence. We are currently testing *C. elegans* exposed either to *C. difficile* spores or to culture supernatants. There are effects in survival rate, but no effects on morphology or behavior of animals (movement).
An important achievement of the project is establishment of widely accessible internet database of *C. difficile* ribotypes. Ribotyping is a standard typing technique used worldwide for *C. difficile*. It is easy and low cost method, however the standard agarose gel based analysis introduced fair amount of errors in typing and exchange of reference strains was absolutely necessary. The modified typing method (capillary gel electrophoresis-based PCR-ribotyping) in combination with web based analysis tool can now be used by laboratories for analysis, type assignment and for the first time for easy comparison of typing results.This will improove the understanding and control of the spread of hypervirulent strains.

Name	**Matthias Maass**
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Title of the project	**Transcriptome-based Monitoring and Eradication of Chronic Chlamydial Infection - ChlamyTrans**
Title of your presentation	Transcriptome-based Monitoring and Eradication of Chronic Chlamydial Infection - ChlamyTrans

ChlamyTrans will use data from two complementary genome-wide transcriptomics approaches to identify commercially exploitable diagnostic tools and drug targets for monitoring and eradicating chlamydial infections. Base is to construct cloned ORFs (ORFeomes) of *C. trachomatis* and *C. pneumoniae* and to identify interfering peptides (interactomes) for deriving therapeutic small molecule inhibitors via a high-throughput robotics platform. In the complementary approach, *in vivo* genome-wide transcriptional profiling of *C. trachomatis* and of *C. pneumoniae* will deliver a definition of the highly expressed chlamydial genes that are needed for *in vivo* survival of the persistent state and overall maintenance of chronic infection. Both approaches will continuously deliver potential drug target proteins, infection markers and inhibitory peptides for validation of clinical and diagnostic relevance in established *ex vivo* and *in vivo* models of chlamydial infection. Some delays in setting up the consortial structure have been encountered due to funding payment in Austria having been delayed to November 2009. Current achievements are:

- Production and subsequent analysis of *C. pneumoniae* and *C. trachomatis* genomic DNA for suitability of full-length gene isolation (evaluation of overall DNA quality, design of specific primers for randomly chosen representative chlamydial genes: successful detection of all probes)
- Design of ~4000 PCR-primers for gene amplification.
- RNA extraction protocol to obtain chlamydial mRNA from *in vivo* infection to analyze *C. trachomatis* gene expression.
- Sample bank of *C. pneumonie* infected materials made available for *in vivo/ex vivo* infection studies
- *C. trachomatis* sample collection has begun (cervical swab for culture and genotyping of the bacterium, another cervical swab for *C. trachomatis* NAA and RNA extraction, a serum sample for antibody determinations and whole blood for DNA extraction).

Understanding the genetic susceptibility to, monitoring, terminating and, ultimatively, preventing the devastating sequelae of chronic infection as well as developing the commercial tools to do so in a novel approach of applied transcriptomics are the aims of ChlamyTrans.

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Title of the project	**Mechanisms and modulation of innate immune responses to Streptococcus pneumoniae and S. pyogenes**
Title of your presentation	Type I interferon response upon infection with *Streptococcus pyogenes*

Type I interferons (IFNs) are powerful modulators of the host defense to infections. The role and the mechanisms of induction of type I IFNs during infections with Gram positive remain poorly understood. Our studies of the responses of the innate immune system to infection with the Gram-positive extracellular human pathogen *Streptococcus pyogenes* revealed that murine bone marrow-derived macrophages as well as conventional dendritic cells produce IFNbeta, the primary type I IFN, in response to *S. pyogenes*. In addition, infection models assessing the contribution of innate immune system revealed that animals lacking type I IFN signaling are more susceptible to *S. pyogenes* than wild type animals. Detailed mechanistic analysis revealed that bacterial RNA plays an important role in the induction IFNbeta. However, the identity of the host cell receptor is currently still unclear since we ruled out the involvement of the majority of the known innate immune receptors. Details about our mechanistic studies as well as the differences between macrophages and conventional dendritic cells with regard to the induction of IFNbeta will be presented at the meeting.

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Title of the project	Pathogen-host metabolomics and interactomics (Pathomics)
Title of your presentation	Pathogen-host metabolomics and interactomics in *Chlamydiae* and *Pseudomonas aeruginosa*

Bacterial protein secretion represents a key mechanism for infection and pathogenesis and enables the modulation of infected hosts by pathogenic bacteria. The diagnostic and therapeutic potential of this process is not limited to the secretion machineries and the segregated proteins themselves, but includes the largely unknown effects of these proteins on the host cells. To exploit this potential, we investigate the host-pathogen protein-protein interactomes of human pathogens and their influence on the metabolic system of the host cells.

In this project, we have established a powerful consortium of young investigators and established researchers, bringing together expertise from biology, chemistry, analytics and bioinformatics. We are studying the molecular basis of pathogen-host interactions by applying integrative and multidisciplinary research approaches to two different classes of pathogenic bacteria, sharing a common principle of host-pathogen interaction: the secretion of effector proteins into their host cells. *Pseudomonas aeruginosa* is a gram-negative bacterium and an opportunistic human pathogen that causes severe infections in immune compromised individuals. *Chlamydiae* are gram-negative bacteria and obligate intracellular human pathogens, causing a broad spectrum of diseases like trachoma, sexually transmitted disease, infertility and community-acquired pneumonia. High prevalence of infection and disease, currently limited diagnostics and unspecific therapy are common to these pathogens. With the aim of developing novel biomarkers and strategies for diagnostics and therapeutics, we are investigating three complementary aspects of bacterial secretomes from genomic, proteomic and metabolomic perspectives:

1. Investigation of the transcriptional context of the secretomes: The assembly of secretion machineries and translocation of effectors require precise spatial and temporal control of gene expression. Therefore we are investigating the transcriptional and regulatory context of the bacterial secretomes by deep sequencing of transcriptomes under different conditions. During the first year of the project, protocols for mRNA extraction and processing have been developed and optimized for *Chlamydiae* as well as *Pseudomonas aeruginosa*. The extracted mRNA of first samples is currently sequenced in a comparative evaluation run in which we test the Illumina and SOLiD next generation sequencing platforms. Parallelizing the sequencing of transcriptomes, we are developing computational methods for the filtering and normalization as well as the integrative interpretation of transcriptomic raw sequence reads. These efforts are complemented by the further characterization of the Type III secretomes. In order to demonstrate the injection of *Pseudomonas* effectors into the eukaryotic cells, we have set up a translocation reporter based on a short phosphorylable tag called GSK. We first tagged a known type III effector and constructed a fusion protein. When translocated into host cells, the tag is phosphorylated and specifically detectable by a GSK phospho-specific antibody. The recognition of Type III effectors by their N-terminal signal peptides has been investigated in an *in silico* model that now allows the sequence based prediction of Type III secreted proteins.

2. Identification and characterization of the host-pathogen protein-protein interactomes: On the molecular and cell biological levels, the specific effect of secreted proteins onto the infected host cells is so far unknown for most effectors. Investigating the molecular crosstalk of pathogen and host cell through interaction proteomics, we decided to initially focus on selected pathogenicity factors from *Pseudomonas aeruginosa* and *Chlamydiae*. To circumvent overexpression induced aggregation problems, we also cloned N-terminal truncations of effectors. PCR products of the selected effectors are cloned into a Gateway Donor vector and subsequently into a GS-TAP vector. All the created vectors have been tested by restriction digests and have been sequenced to verify the integration of the correct construct. We are currently testing all the vectors for expression in transient transfection in Hek293 cells. Once all the vectors are at hand we will start producing retrovirus to create HelaS3 cell lines stably carrying the effectors proteins. These cells will finally be used for Tandem Affinity purification (TAP) and further analysis of co-purified interacting host proteins via mass spectrometry (MS). Additionally, protein-protein interactions of *Pseudomonas aeruginosa* PA14 with host cells are currently studied using Yeast two-hybrid (Y2H) screens and by investigating the phenotypic response of *Pseudomonas* effectors in yeast. Bioinformatics approaches are developed for the identification of putative bacterial effectors by the phylogenetic profiles of their protein domains.

3. Analysis of the host-pathogen metabolomes: The systematic, qualitative and quantitative study of small-molecule metabolites provides a complementary view to the physiology of cells, compared to transcriptomics and interactomics. The first samples from *C. pneumoniae* infected HEp-2 cells were analyzed in the Ion cyclotron resonance Fourier transform mass spectrometry (ICR FT/MS). The aim was to investigate the reliability of the obtained data. The measurements showed good repeatability. Hierarchical cluster analysis was used to compare the spectra of infected and non-infected cells and hereby clustering in the growth time was observed. For the metabolite annotation our web interface MassTRIX was used. We further improved the tool allowing faster comparisons. These three complementary approaches are performed in a very close interconnection between the groups of the consortium and between experimental and computational approaches.

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Title of the project	METAGUT: Development, prevention and early diagnostic detection of Clostridium difficile associated colitis
Title of your presentation	METAGUT: Development, prevention and early diagnostic detection of Clostridium difficile associated colitis

Pseudomembranous colitis (hereinafter PMC) is an infectious disease of the human colon mostly caused by Clostridium difficile. The disease is characterized by diarrhea, fever and abdominal pain. A main factor underlying the development of pseudomembranous colitis is the use of broad-spectrum antibiotics, such as clindamixcin or cephalosporins, which lead to an altered intestinal microflora allowing Clostridium difficile bacteria to be activated or overgrow. The colitis itself is caused by special toxins of Clostridium difficile (toxin A, B). The diagnostic tests are based on both the culture of the bacterium and the detection of toxin A.

In addition to the obvious burden to the many individual patients, PMC generates tremendous costs in the health systems of most of the industrialized countries. Even in the hospital environment, pseudomembranous colitis requires isolation of patients and complicates clinical management of disease ending in higher hospital residences and treatment efforts. Moreover, resistance of Clostridium difficile strains to specific antibiotic therapy (i.e. metronidazole or vancomycin) with severe – and sometimes lethal - course of disease is a growing problem of the last years.

Diagnosis and treatment of pseudomebranous colitis are laid down in clinical guidelines and are backed by clinical randomized studies, but little is known about the development of the disease. In ailments such as obesity and Crohn disease, the ethiology and severity of disease has been shown to be directly related to the composition and functional status of the gut microbiome. In PMC, it is hypothesized that alterations of the composition of the intestinal microflora lead to competition of microorganisms for space and nutrients. Revealingly, probiotic preparations seem to be effective in the prevention and of pseudomembranous colitis. However, as yet, there is no clear concept about the pathophysiology and pathogenesis of pseudoembranous colitis, nor a through understanding of the mechanisms and interactions this serious disease.

This project thus aims:

a) to generate insights into the onset and development of Clostridium difficile-associated pseudomembranous colitis and of its relation to (altered) gut microflora in humans, and

b) to translate this knowledge into strategies for early diagnosis and prevention.

This will be done through an integrated approach whereby the composition and metabolic status of the gut microbiome of healthy individuals and from patients with broad-spectrum antibiotic therapy and pseudomembranous colitis will be thoroughly characterized and compared. The composition of the intestinal microflora (16S-based) before, during and after manifestation of colitis will be determined in patient materials provided by partner 1. Assessment of the differences on the microbial composition of faeces and intestinal mucosa (Partners 1 and 4a) will enable to assess in how far the use of non-invasive sampling procedures (stool) provides samples that reflect the conditions in the gut as obtained by invasive sampling (endoscopy). The (pyro-)sequencing of selected metagenomes of healthy individuals and patients with broad spectrum antibiotic therapy and pseudomembranous colitis at different stages of disease (partner 4a and 4b, which can be possibly subcontracted to 4a if the regulations so require), coupled to the in-depth analysis of the metabolic profiling through a high-throughput array screening method (partner 3), meta-proteomics (partner 2) and meta-transcriptomics (partners 1 and 5), and assisted by a comprehensive bioinformatics analysis and reverse engineering of the high-throughput information produced (partners 2, 4a and 6), will provide compositional and functional signatures associated to pseudomembranous colitis. The functional caracterisation of the cell-wide responses of C. difficile under the different settings/conditions (partners 1,2, 3, 4 and 6) will provide a basic blueprint of the clostridial infection and solid framework with which to study and to generate new hypotheses capturing the key features underlying this serious condition. In addition to the wealth of knowledge generated on the mechanisms underlying the disease, this will enable the identification of markers for early diagnosis and will lay the foundation for the development of prevention and intervention strategies to fight *C. difficile*-associated PMC. This multidisciplinary project builds on the joint efforts of a major clinical hospital, one reputed university, two prestigious research institutions and three leading industrial partners (SME's).

The ultimate goal of the proposed network is to characterize the composition and metabolic/functional status of intestinal microflora from the initial, non-diseased stage towards Clostridium difficile-associated pseudomembranous colitis in the human system, providing thereby understanding of this troublesome and costly disease and paving the road for the early diagnosis and development of effective prevention and intervention strategies.

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Title of the project	Identification of hot spots of divergence and rapidly changing genes within Shiga toxin-producing *Escherichia coli*
Title of your presentation	Identification of hot spots of divergence and rapidly changing genes within Shiga toxin-producing *Escherichia coli* (Coordinator`s project presentation)

In this project, we will determine the progression by which genomic loci were acquired, lost, or altered within a well-defined clade of highly pathogenic Shiga toxin-producing *E. coli* (STEC). Such strains have recently emerged as causes of bloody diarrhoea and of life-threatening hemolytic uremic syndrome (HUS), the major cause of acute renal failure during childhood. Therefore, we will develop a roster of loci that are found in human pathogens within this clade, and determine if they are present also in environmental STEC isolates and in adherent invasive *E. coli* (AIEC) isolates from Crohn's disease. To identify rapidly evolving genes (protein coding and promoter regions) or chromosomal domains we will (i) compare the divergence rates between the different strains by SNP analysis and we will (ii) determine if there are regional hot spots of divergence or rapidly changing genes. This analysis involves tracking the rate of nucleotide substitutions along the length of the genome to determine if the divergence rate is uniform throughout the genome. Furthermore, (iii) pair-wise comparisons and multiple alignments of orthologous genes will be performed at nucleotide and amino acid level to characterize the evolution within rapidly changing genes. In addition, (iv) we will study global transcriptional changes focused on the transcriptional regulatory network within a well-defined clade. Multiple virulence factors are controlled by some specific transcriptional factors which expression can be different between bacteria inside a clade. Finally, (v) we will investigate the influence of these genes to the host-pathogen interaction in functional assays (e.g., cytotoxicity assays, complement activation, adherence, invasion) to confirm the genomic heterogeneity as reason for the variable pathogenicity.

POSTERS 1st call projects

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Title of the project	Deciphering the intersection of commensal and extraintestinal pathogenic *E. coli*
Title of your presentation	Non-immune and immune haemolysis may both contribute to disease progression in infection by alpha-haemolysin producing *Escherichia coli*

Levente Emődy1, István Bátai2 and Monika Kerényi1

Introduction: Alpha-haemolysin of *E. coli* is the prototype of the RTX (**R**epeat in **T**o**X**in) toxin family. It has long been known to contribute to the virulence of extraintestinal pathogenic *E. coli* (ExPEC) strains. In the compromised host such infections may present with jaundice due to intravasal haemolysis. Here we present evidence that not only toxin mediated lysis of erythrocytes but also immune haemolysis may be involved in disease history.

Results: Serum samples from the patients exhibited high-titre anti-haemolytic activity *in vitro*, and the titres correlated with the gravity of infection. Despite the presence of these neutralizing antibodies and repeated transfusions with erythrocyte mass the haemolytic symptoms progressed in the patients. As complement is not present in the *in vitro* assay but obviously present in the patients' circulation we assumed that complexes formed by haemolysin and anti-haemolysin might have been deposited to the target erythrocytes, and induced an immune complex mediated haemolysis through activation of the classic complement pathway. We modified the *in vitro* neutralization assay by adding either active or inactivated complement. As expected, in the presence of active complement the neutralizing effect of the anti-haemolytic sera was entirely abolished while inactivated complement did not affect the reaction at all.
The ability of alpha-haemolytic *E. coli* to cause intravasal haemolysis was proven in intravenous and intranasal mouse models using isogenic haemolytic and non-haemolytic bacterial clones of urinary isolates from haemolytic patients. Haemoglobinuria and acute haemorrhagic lung oedema pointing to *in vivo* haemolysis was experienced only after infection with the haemolytic counterparts.

Conclusions: Transfusion with erythrocyte mass is not a remedy for the haemolytic patient as the transfused cells also suffer complement mediated lysis. Prompt adequate antibiotic treatment may be life saving by abolishing bacterial multiplication and this way further production of alpha-haemolysin. In the most severely ill patients alpha-haemolysin producing *E. coli* was present also in the faecal samples. Harbouring such strains in the gut exposes the compromised host to a special risk for infection with haemolytic complications.

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Title of the project	Deciphering the interface of commensal and extraintestinal pathogenic *Escherichia coli*
Title of your presentation	DNA Microarray for the Phylogenetic Classification of Pathogenic *Escherichia coli* Strains

The species *Escherichia coli* comprises not only commensal strains but also many pathogenic variants. A group of infection causing *E. coli*, the extraintestinal pathogenic *E. coli* (ExPEC), is responsible for a variety of diseases such as urinary tract infections, septicaemia or neonatal meningitis. In consequence, the treatment of ExPEC infections results in high health care costs.

Amongst different methods, phylotyping can be used for the assessment of the pathogenic potential of an isolate, because independent studies have shown that there is a correlation between the phylogenetic background and the pathogenicity of a certain strain. The species *E. coli* can be divided into the ECOR groups A, B1, B2, D and E whereas ExPEC strains mainly belong to group B2 and, to a lesser extent, to group D. In consequence, it is mandatory to employ diagnostic tools for the phylotypic classification of *E. coli*. The gold standard in the field of phylotyping is certainly multilocus sequence typing (MLST), which is based on the complete sequencing of seven housekeeping genes. This method produces very accurate results but it is, however, expensive and time consuming. In this context, phylotyping by SNP detection promises to be a competitive alternative. Hommais and coworkers (2005) have shown that the analysis of 13 SNP (single nucleotide polymorphism) in five housekeeping genes is sufficient to reconstruct the ECOR groups correctly.

In this study, the system by Hommais *et al.* (2005) was successfully transferred to a diagnostic DNA Microarray platform. In this context, the establishment of this assay included the optimisation of capture probes and the validation with different reference strains. A reduction of assay costs was possible by a multiplex amplification and fluorescence labeling of all target regions. Consequently, all significant false-positive signals due to unspecific crosshybridisations were eliminated. Finally, the analysis of 22 blinded clinical samples was in concordance (> 80 %) with the reference method developed by Clermont and coworkers (2000). These results are consistent with the findings by Hommais *et al.* (2005). In combination with other diagnostic biochips for the detection of antibiotic resistances or pathogenic traits, this novel microarray represents a powerful tool for epidemiologic studies and diagnostics.

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Title of the project	1st call: Deciphering the intersection of extraintestinal pathogenic and commensal *Escherichia coli*
Title of your presentation	Lack of major immune-determinants on enteric bacteria results in enhanced cross-protective capacity of live attenuated mutants

Infections caused by *Enterobacteriaceae* result in a wild variety of common diseases. The tendency of several species to develope resistance to antibiotics, as well as their ability to cause outbreaks make a vaccine desirable. Development of broad protective vaccines is hindered mainly by the heterogenicity of the immunodominant antigens (usually the serotype determining antigens). As enterobacteria express uniform/conserved epitopes in a huge number as well, it seems to be an attractive approach to engineer vaccine strains capable of eliciting a protective immune response against these shared epitopes, hence being cross-protective.

Russo et al. proved, that a mutant of extraintestinal pathogenic *E. coli* (ExPEC) lacking the capsular and O-antigens could provide an improved humoral immune response against the non-capsular and non O-antigen epitopes than the wild type strain. Furthermore, the antibodies provoked by this mutant could enhance the neutrophil-mediated bactericidal activity agains heterologous strains as well. Nagy et al. constructed a „gently rough" mutant of *Salmonella enterica* serovar Typhimurium (Δ*rfaH)* resulting in diverse length of the LPS molecules and proved that immunisation with this strain could elicit improved immune response against shared antigens and triggered cross-protection in a murine model.

Based on these results we constructed a Δ*rfbF* mutant of a parental *Shigella flexneri 2a* strain devoid of the O-antigen and selected a variant of the mutant lacking the invasion plasmid (hence the highly immunogenic invasion plasmid antigens – Ipa-s as well). The immunogenic potential of the strains was examined upon mucosal immunisation using the mouse lung model.

Immunisation studies provided evidence that both the invasive and non invasive strains elicited significant protection against a homologous challenge. Determination of the serum and mucosal antibody levels attested that the rough non-invasive "double" mutant not only provoked significant anti-*Shigella* titres, but also raised antibodies against heterologous *Shigella* serotypes as well as other representatives of the family *Enterobacteriaceae*. Indeed, the double mutant provided high protection against heterologous strains, which implies that the existence of protective shared antigens is likely. Identification of these conserved antigens could be of major importance in cross-protective vaccine development.

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Title of the project	UTI-Interference
Title of your presentation	Host driven adaptation of asymptomatic bacteuria *Escherichia coli* strain 83972

Escherichia coli, the major cause of uncomplicated symptomatic urinary tract infections (UTI), may also cause asymptomatic bacteriuria (ABU), i.e. a carrier state without symptoms. In contrast to acute pyelonephritis isolates, many ABU strains fail to express functional virulence factors, suggesting that the difference in virulence might explain the severity of infection. The molecular mechanisms that underpin ABU are not well understood. Bacteria associated with ABU are often left untreated and may even be beneficial in preventing symptomatic infection by more virulent organisms (bacterial interference). In this study the strain 83972, that was originally isolated from a young female with long term ABU, was used for deliberate colonization of patients with recurrent UTI. To study adaptational changes upon long term growth in the bladder, consecutive re-isolates were analyzed with regard to changes in their transcriptome and proteome as well as genome rearrangements. The results obtained were compared with those from re-isolates from a control experiment, in which strain 83972 was propagated over two months in pooled human urine under *in vitro* conditions. This is the first genome-wide example of bacterial evolution in human hosts and provides the surprising insight that individual hosts essentially will imprint their microflora through alterations in metabolic and fitness genes, and in pleiotropic regulators of bacterial gene expression.

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Title of the project	"Deciphering the interface of commensal and extraintestinal pathogenic *E.coli*"
Title of your presentation	Comparative genetic analysis of avian extraintestinal and intestinal *Escherichia coli* strains isolated in Hungary

Extraintestinal pathogenic Escherichia coli (ExPEC) possesses virulence traits that allow it to invade, colonize, and induce disease in different organs outside of the gastrointestinal tract thereby causing major losses in the poultry industry all over the world. The origin of ExPEC strains is assumed to be the intestinal tract, which is normally colonized with an abundance of strains of commensal, intestinal E. coli (IntEc), which may also have some basic traits of pathogenicity.

In an attempt to search for genetic relationship/differences between extraintestinal and intestinal E. coli of avian origin we have tested 71 ExPEC isolated from liver, lung or bone marrow of dead chicks and turkey poults that died due to colibacillosis, and 43 E.coli were isolated from caeca of birds of similar age that died from the same farm, and were epidemiologically unrelated to the death of colibacillosis.

First we performed a pilot study on 6 ExPEC and 6 IntEc and screened for the presence of 73 different virulence-related genes by PCR. Besides the phylogenetic groups of the strains have been determined by the triplex PCR of Clermont et al (2000).

As a result, the ExPEC isolates seemed to be characterized by the *cdtIV, fyuA, vat* and *pic* genes.

One ExPEC strain of O1:K1 seemed to resemble the prototypic avian ExPEC, belonging to phylogenetic group B2, harbouring a P-fimbrial determinant, the PAI marker malX and a K1 capsule determinant.

In further studies we have focused on the above virulence and phylogenetic PCRs on all ExPEC and IntEc. Results indicated that the PAI marker malX was equally rare (approx 4%) in both groups, while *pic* gene and the cytolethal distending toxin gene *cdtIV* were exclusively present in ExPEC (12.7% and 0.6 % respectively). The latter 4 ExPEC strains seemed to represent two new clones (O53 and O115). Furthermore, the *vat* gene was twice more frequent in ExPEC as compared to IntEC (24% vs 11.6%). In general, the various intestinal and extraintestinal E. coli virulence traits were rare in both groups.

On the other hand, most of the strains in both groups had a colicin plasmid or derivatives thereof (genes *iut, iss*).

Regarding phylogenetic groups, the distribution of the two groups of strains was quite similar (Table 1), except group "A" (40.8% among ExPEC), and Group "B1" (41.9% among IntEC).

Table 1. Phylogenetic groups of avian ExPEC and IntEC strains as determined by PCR

Origin	No of strains	A	B1	B2	D
ExPEC	71	29 (***40.8%)***	10 (14%)	11 (15.5%)	21 (29.6%)
IntEc	43	10 (23.2%)	18 (***41.9%)***	6 (14%)	9 (20.%)
Total	**114**	**39 (34.2%)**	**28 (24.6%)**	**17 (14.9%)**	**30 (26.3%)**

It is concluded that some intestinal E. coli (IntEc) may have a closer genetic relation to most ExPEC strains while others seem to be further away from the chance to develop into a matured ExPEC.

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Title of the project	European Initiative to Fight Chlamydial Infections by Unbiased Genomics
Title of your presentation	Developing Electric Cell-Substrate Impedance Sensing -method to study *Chlamydia pneumoniae* infection in HL-cells.

Chlamydia pneumoniae is a worldwide pathogen that is difficult to eradicate completely from human host. Normally it has a two stage developmental cycle with an infectious elementary body (EB) and a dividing reticulate body (RB) stage, as well as a third developmental stage (aberrant chlamydia) when confronted with growth limiting factor (1). The complexity of these stages along with the intracellular nature makes *C. pneumoniae* infection difficult to monitor in real time and thus a challenging target for drug development (2).

Conventional label-dependent methods to monitor chlamydial infection are based on fixed cell cultures which can be used to interpret results from isolated time points (3). Even increasing the number of time points only gives an estimate of the infection process and increase tremendously the workload.

We have aimed to develop an easy, noninvasive, label-free, “hands of” -method to follow the host cell exit process of *C. pneumoniae* in real time and to study the effect of selected anti-chlamydial substances on the time and rate of host cell exit of *C. pneumoniae. E*lectric *C*ell-Substrate *I*mpedance *S*ensing (ECIS) is a suitable method to track the attachment behavior of mammalian cells (4) and thus it can be used to monitor also the detachment of host cells caused by intracellular pathogens exiting the host (5).

Preliminary data from HL-cells alone and HL-cells infected with *C. pneumoniae* show the suitability of ECIS to track the host cell detachment caused by *C. pneumoniae* and thus the end stage of chlamydial developmental cycle. Results and the benefits and drawbacks of this method will be discussed.

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Title of the project	Exploring Protein Secretion within the bacterial biofilm matrix. Acronym: EPS-Matrix
Title of your presentation	Protein-mediated biofilm development in *Staphylococcus aureus.*

Despite the general agreement that the exopolysaccharides plays a key role in biofilm development, increasing number of evidences indicate the existence of proteinaceous-dependent biofilm matrix. The existence of biofilm matrixes of different composition suggests that each matrix might confer different properties to the embedded bacteria. Because the proteomic analysis of the exopolysaccharide matrix of *Salmonella enteritidis* and *Staphylococcus aureus* revealed the presence of very few proteins, whose presence is not specific for biofilm matrix, we focused our efforts to investigate protein-mediated biofilm development in *S. aureus*. The ultimate goal will be to understand the influence that biofilm matrix nature (polysaccharidic or proteinaceuos) has on embedded bacteria. For that, we first analyzed the protein-mediated biofilm matrix developed by some *S. aureus* strains in the absence of ArlRS two-component system. To identify the protein component responsible for the PIA/PNAG-independent biofilm development in arlRS mutants, fixed bacterial cells were digested with trypsin to yield a peptide mixture that was fractionated and analyzed by 2DnLC coupled to electrospray ionization and mass spectrometry. The results of the study revealed that overexpression of Protein A induced intercellular aggregation and biofilm development. Exogenous addition of purified protein A or supernatants containing secreted protein A to growth media induced biofilm development, indicating that, at least, protein A can promote biofilm development without being covalently anchored to the cell wall.
On the other hand, we investigated a clinical *S. aureus* MRSA strain able to switch between proteinaceous and polysaccharidic biofilm matrix depending on the environmental conditions. Systematic
disruption of each member of the LPXTG surface protein family identified fibronectin-binding proteins (FnBPs) as components of a proteinaceous biofilm formed in Trypticase soy broth-glucose, whereas a PIA/PNAG-dependent biofilm was produced under osmotic stress conditions. The induction of FnBP levels due to the activation of a LexA-dependent SOS response or FnBP overexpression from a multicopy plasmid enhanced biofilm development, suggesting a direct relationship between the FnBP levels and the strength of the multicellular phenotype. Interestingly, scanning electron microscopy revealed that cells growing in these protein-mediated biofilms formed highly dense aggregates without any detectable extracellular matrix, whereas cells in a PIA/PNAG-dependent biofilm were embedded in an abundant extracellular material.
Finally, we compared the contribution of protein-mediated biofilm with respect to exopolysaccharide-mediated biofilm to colonization of subcutaneously implanted catheters. Unexpectedly, the results revealed that both Protein A and FnBP mutants displayed a significantly lower capacity to develop biofilm on implanted catheters than their corresponding isogenic PIA/PNAG-deficient mutants. These results strongly suggest that protein-mediated biofilms might play a more relevant role than exopolysaccharide-mediated biofilm in the colonization of the implanted devices.

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Title of the project	FunPath
Title of your presentation	*Candida glabrata* persistence and immune evasion strategies

Although *Candida glabrata* is an emerging nosocomial pathogen which can cause life-threatening systemic infections in the human host, the fungus has been described as surprisingly benign in mice. As part of the FunPath project, we have established a mouse model of *C. glabrata* infections. In this model, live *C. glabrata* yeasts could not only be re-isolated from leucopenic and corticosteroid-treated mice, but also from fully immunocompetent animals, for at least four weeks after infection. During this period, several clinical parameters, like blood enzyme levels, histology, myelperoxidase and cytokine levels, showed only a very mild immune response of the host. This indicates that *C. glabrata* has developed strategies to persist in the host, possibly by a specific immune evasion strategy.
In fact, *in vitro* experiments showed that *C. glabrata* is able to replicate inside monocyte-derived macrophages (MDM). Fungal cells were taken up readily by phagocytes and the resulting phagosomes initially followed the normal route of maturation as indicated by the presence of the endosomal EEA1 and LAMP-1 markers. However, phagosomal acidification was blocked. Phagosomes containing live, but not heat-killed fungi, showed no active cathepsin-D protease and are not stained by the acidophilic Lysotracker probe. Furthermore, no significant induction of proinflammatory cytokines was observed in MDMs after phagocytosis of *C. glabrata*.
Currently, investigations are underway to determine which fungal factors are involved in the persistence of *C. glabrata* inside macrophages, by using a mutant library and genome wide microarrays developed by FunPath partners.
We also investigated pigmentation as a possible virulence factor of *C. glabrata*. When provided with tryptophan as the nitrogen source, the fungus produces a dark-brown pigment. Transcriptional profiling and mutant library screening indicated that this pigment is a by-product of the Ehrlich pathway. Using targeted deletion mutants and recombinant expressed proteins we show that the aromatic aminotransferase Aro8 is both necessary and sufficient for the synthesis of pigment from tryptophan, by converting the amino acid into the pigment precursor molecule indole pyruvate. Similar to other fungal pigments, such as melanin, the tryptophan-derived pigmentation caused increased survival of *C. glabrata* when the fungus was exposed to UV light or hydrogen peroxide, and has a protective effect against damage by neutrophils.

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Title of the project	Genomic Approaches to Unravel the Molecular Mechanisms of Pathogenicity in the Human Fungal Pathogen Candida glabrata - **FunPath**
Title of your presentation	Comprehensive gene deletion study to identify cell wall organisation and structure in *Candida glabrata*

Ekkehard Hiller[1], Marcel Dörflinger[1], Rebecca Stevens[1], Tobias Schwarzmüller[3], Toni Gabaldon[2], Marina Marcet-Houben[2], Karl Kuchler[3] and Steffen Rupp[1]

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Although *Candida glabrata* has become the second most important pathogenic Candida species, only few of its virulence mechanisms have been identified so far.
To get a more comprehensive idea of the virulence mechanisms of *C. glabrata*, we use wide-ranging gene deletion studies in order to elucidate genes involved in pathogenicity. These studies are undertaken within an ERA-Net consortium, FunPath. Genes coding for predicted proteins of the cell wall, known signalling pathways, membrane-bound receptors, transporters and transcription factors were identified by comparative genome analysis and subsequently deleted (about 500 deletion mutants at present). This library is screened with biological assays, e.g. for strains with altered cell wall stability, stress tolerance, or adhesion.
In a recent study, 39 deletion mutants of possible cell wall integrity influencing genes in *C. glabrata* were investigated concerning their growth behaviour on agar plates with different cell wall stress inducing agents. The plate tests showed different mutant strains being susceptible against cell wall stress conditions, including knock out mutants of the cell wall integrity signalling pathway. Further investigations in adhesion characteristics, cell wall composition and stability and transcriptional profiles were made concerning this pathway for the deletion mutant strains Δ*slg1*, Δ*mid2* and Δ*slt2* to enable conclusions of a functional connection between these genes in *C. glabrata*.
Regarding adhesion on agar plates and on human epithelial cell monolayer, Δ*mid2* and Δ*slt2* showed comparative behaviour to the reference strain, whereas the mutant strain Δ*slg1* was on both surfaces significantly stronger adherent. Similar behaviour could be observed while assaying cell wall stability and composition, with Δ*slg1* having considerably changed properties in comparison to the mutant strains Δ*mid2* and Δ*slt2* and the reference strain.
Comparing the trancriptomes of the deletion strains with the reference strain in the presence or absence of the cell wall stressing substance Congo red showed only very slight changes in the expression pattern of Δ*mid2* and Δ*slt2*, whereas in Δ*slg1* a significantly changed transcriptional profile could be detected.
In summary, it can be concluded that the results of this study can be seen as first step for the characterization of the investigated genes as potential virulence relevant factors and target structures for the later application of antimycotic agents.

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Title of the project	Genomic Approaches to Unravel the Molecular Mechanisms of Pathogenicity in the Human Fungal Pathogen Candida glabrata - FunPath
Title of your presentation	**Transcriptional rewiring of the *Candida glabrata* cell wall biosynthesis *CBK1***

Tobias Schwarzmüller1, Fabian Istel1, Walter Glaser1, Ekkehard Hiller2, Steffen Rupp2 and Karl Kuchler1

Candida glabrata is an opportunistic human fungal pathogen which can cause life-threatening systemic infections in immunocompromised individuals. Screening our *C. glabrata* gene deletion collection identified a Cg*cbk1*Δ strain displaying heavily wrinkled colonies. Cbk1 is the master regulator of the yeast RAM (Regulation of Ace2p transcription factor and polarized Morphogenesis) network and controls polarized growth, cell separation and the induction of daughter cell-specific genetic programs via the transcription factor Ace2. Inactivation of the *C. glabrata* RAM network transcription factor Cg*ACE2* results in a hypervirulent strain defective in cell separation (1).
The lack of the *C. glabrata CBK1* gene results in a similarly severe growth defect. Cg*cbk1*Δ cells are unable to separate properly, form large cell aggregates, and display very large, round cells. Exposure to various cell wall-perturbing compounds results in growth sensitivity of the Cg*cbk1*Δ mutant. Interestingly, transcriptional profiling identified not only genes regulated by CgCbk1 via the transcription factor CgAce2 but also differential expression of *EPA13*, a member of the large family of epithelial adhesins (*EPA*). In addition, CgCbk1 appears to control several amino acid biosynthesis-related and metabolic genes. Calcofluor white staining and cell wall analysis of the Cg*cbk1*Δ cells showed that more chitin and less glucan is incorporated in the cell wall leading to a severely changed wall composition.
The cause for the hypervirulence phenotype of the Cg*ace2*Δ mutant is unknown. We observed that cells lacking either Cg*CBK1* or Cg*ACE2* are not phagocytosed by murine bone marrow-derived macrophages (BMDM) *in vitro*. The inability to internalize the large cell aggregates formed by these mutants is reversed when the cell aggregates are separated into single cells prior to infection or by complementation with the endogenous gene. We suggest that the inability to phagocytose those large cells aggregates and the differences in the cell wall composition may add to changes in the immune response of the macrophages and contribute to the hypervirulent phenotype described for the Cg*ace2*Δ mutant.

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Title of the project	Glycoshield
Title of your presentation	**Tsa1p modulates the cell surface of *Candida albicans***

Tsa1p modulates the cell surface of *Candida albicans*

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The cell wall is the first contact site between host and pathogen and is thus critical for colonization and infection of the host. We have identified Tsa1p (Thiol-specific antioxidant-like protein) as part of the cell wall and within the cytoplasm of *C. albicans*. Tsa1p has been shown to be responsible for several distinct functions, including functions in oxidative stress and genome stability. It does not contain a typical signal sequence for entry into the secretory pathway therefore the mechanism by which Tsa1p is released to the cell surface is unknown. We could show that localization of Tsa1p to the cell wall is determined by different parameters. In previous experiments Tsa1p could only be detected at the cell surface in hyphae-inducing media indicating a morphology-dependent localization of Tsa1p to the cell surface. In addition, time course experiments showed a temporary translocation of Tsa1p to the cell surface in yeast form cells after transferring stationary phase cells to media independently if the media is fresh or conditioned (medium from o/n culture). Increased Tsa1p cell surface localisation could also be triggered by applying oxidative stress (H_2O_2), addition of reducing agents like DTT or ß-Mercaptoethanol and incubation with CO_2. However, in yeast form cells Tsa1p can only be detected at the cell surface during the lag and log-phase of growth. This indicates a connection with quorum sensing. Indeed addition of farnesol to YPD medium results in stronger and longer lasting accumulation of Tsa1p to the cell surface.
To check on regions within *TSA1* that are required for localization, we deleted the C-terminal 12 amino acids of Ca*TSA1*. From its human homologue it is known that a signal for membrane localisation resides in the C-terminal part. Additionally, the cysteines of the two active sites of Tsa1p were substituted by serines to check their role in Tsa1p function and localisation.
All mutants showed sensitivity to oxidative stress (H_2O_2) like the delta-*TSA1* strain. Cell surface localization of Tsa1p in the active site mutants is strongly reduced compared to the wildtype indicating that an active form of Tsa1p is needed for localization of Tsa1p to the cell surface. However, in the mutant strain containing the *TSA1* copy with deleted C-terminus Tsa1p is still able to localize to the cell surface, indicating that the C-terminus is not responsible for its localization in *C. albicans*.
In addition, all mutants show a reduced survival rate when exposed to neutrophils and also have a higher beta-glucan exposure at the cell surface of blastospores compared to the wildtype. This confirms that the cell wall composition in these mutants is altered and that Tsa1p has an important role in maintaining the cell wall composition.

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Title of project: Glycoshield

Title of the poster :

Study of the *Candida albicans IFF* genes family during interactions with the host.

Candida albicans is the fungal pathogen most commonly isolated in humans. It is a normal component of the oral cavity, the gastrointestinal tract and the vaginal environment, but this organism can become an opportunistic pathogen. This pathogen commonly cause superficial infections but can also cause more serious systemic infections in immunocompromised patients. Thereby this is a good model to study the switch between the non-pathogenic and pathogenic states.

Our team is particularly interested in the cell wall proteins because of their localization directly in contact with the host and its environment. Several cell surface mannoproteins have been already involved in virulence traits (adhesion, invasion and immunomodulation). We know that the main class of these proteins is represented by the GPI anchored proteins, the focus of the team. 115 putative GPI anchored proteins were identified *in silico* in the whole *Candida albicans* genome (RichardML & Plaine A, EC2007). The biggest family of these GpiPs is represented by the *HYR1* homologs and the *IFF* (IPF family F) genes. We initiated the study of the entire IFF family during host-pathogen interactions using a ΔΔ*Iff* mutant library. A mutant for each gene was constructed or gathered from other laboratories, except for *Iff3* and *Iff9* null mutant that we were unable to obtain. We only produced the heterozygotes for each gene. After testing different stressing and filamentation inducing conditions (CFW, SDS, pH, Serum, Spider, etc), we did not identify new mutant affected by the deletion except for the already known *iff11*-/-. The absence of a common known function and the possible functional redundancy within the family, prompt us to monitor the expression of each gene using real time quantitative PCR in a wild type and each mutant backgrounds. These experiments were done in normal lab growth conditions and in conditions close to *in vivo* growth (adhesion to HeLa epithelial cells, interaction with macrophages). We observed that *IFF7* and *IFF8* were induced in several iff-/- strains during adhesion to HeLa cells. *IFF3* and *IFF9* were also induced in different *iff*-/- null mutants but independently to the conditions tested (adhesion on plastic or HeLa cells) suggesting that *IFF7*, 8, 3 and 9 might compensate the lack of other members of their family. Finally, we also tested the mutants phenotype during these interactions (adherence to HeLa and sensitivity to phagocytosis) but no phenotype was observed. Further work is needed to understand what is the function of these proteins, but the compensatory regulations observed might give us entry points to unravel this.

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Title of the project	Surface modulation of the fungal & Host response using a genomic approach - Fungal Glycoshield
Title of your presentation	Transcriptional regulation of phosphopeptidomannan and phospholipomannan β-1,2 mannosylation processes

Transcriptional regulation of phosphopeptidomannan and phospholipomannan β-1,2 mannosylation processes

Chantal Fradin, Masset Annick, Céline Mille and Daniel Poulain.

Among the established factors contributing to *C. albicans* virulence are cell wall oligomannosides with an unusual type of linkage, β-1,2 oligomannosides (β-1,2 Man), rare in the living world. During *C. albicans* infection, β-1,2 Man play an important role in host/pathogen interactions by acting as adhesins and by interfering with the host immune response. These particular oligomannosides have been found to be associated with the cell wall phosphopeptidomannan (PPM), phospholipomannan (PLM) and mannoproteins. If it's clear that these glycoconjugates are responsible for the cell wall expression of β-1,2 Man, little is known about their respective contribution in the global expression and modulation of these oligomannosides at the cell surface. Several studies have indeed shown that β-1,2 oligomannosides expression is heterogeneous at the cell surface, varies depending on *C. albicans* strains and can be modulated by several environmental factors (e.g. pH, temperature,…).

We have identified a family of 9 genes designated *CaBMTs* involved in *C. albicans* β-1,2 mannosylation. Six of these genes encode enzymes responsible for β-mannose transfer on PPM (*BMT1-4*) and PLM (*BMT5-6*). In order to determine if regulation of PPM and PLM β-1,2 mannosylation occurs, even partly, at the transcriptional level, *BMT1-2,5-6* expression was compared to detection of β-1,2 Man epitopes on PPM and PLM. *C. albicans* serotype A and B strains with various amount of β-1,2 Man were used in this study and grown in several conditions known to differently affect PPM and/or PLM β-1,2 mannosylation. Despite a weak expression of *BMTs*, there was a good correlation between regulation of *BMT* transcripts level and modulation of β-1,2 Man association to PPM and PLM. Furthermore, as expected, PPM and PLM β-1,2 mannosylation processes were differently regulated suggesting that they are not under the same control. The data also show that, as already suggested by earlier studies, antigen 6 that corresponds to β-1,2 Man attached to PPM acid stable fraction is not specific to serotype A strains but is rather synthesized, for the first β-mannose attached, by an enzyme, Bmt1, encoded by a gene whose expression is repressed in serotype B strains in some growth conditions.

The work presented here suggests the existence of a transcriptional regulation process controlling the amount of β-1,2 Man at the cell wall surface. Furthermore, transcriptional analysis of all *BMTs* in *in vitro* conditions and infection models will be necessary to go further into the complex mechanism of β-1,2 Man expression.

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Title of the project	Glycoshield: Surface modulation of the fungal & host response using a genomic approach
Title of your presentation	Functional analysis of new elements of the SVG and HOG pathways in the fungal pathogen Candida albicans

Previous data of our group showed the essential role that Sho1 and the Msb2 proteins have in morphogenesis, cell wall biogenesis and stress. Both proteins are essential for the activation of the Cek1 MAP kinase but not for the Hog1 phosphorylation under osmotic stress. The aim of this work has been the characterization of new elements of the HOG pathway. For this purpose we have deleted *OPY2* gene in different backgrounds given the role that Opy2 has in *Saccharomyces cerevisiae* within the HOG pathway. Our results demonstrate that Opy2 has a role in the cell wall biogenesis, since 1) *opy2* mutants are sensitive to different cell wall inhibitors (such as congo red or calcofluor white) and 2) collaborates with Sho1 in transmitting the signal to the MAP kinase Cek1 under certain conditions (such as the resumption from stationary phase of growth or the addition of cell wall inhibitors). Opy2 has, however, no apparent role in the response to oxidative stress response. Deletion of *OPY2* in the triple mutant *ssk1 sho1 msb2* does not blocked the activation of Hog1 under osmotic stress although the osmosensitive phenotype of these strains. These results suggest that neither Sho1 nor Msb2 nor Opy2 are important for transmitting the signal under osmotic stress to Hog1, an important difference to what occurs in *S. cerevisiae*. In order to identify additional upstream elements of the HOG pathway we performed a genetic screening in *S. cerevisiae* using a *Candida albicans* genomic multicopy library. The osmosensitive ssk2*/22 hkr1 msb2 sho1* and *ssk2/22 hkr1 msb2* mutants were selected as genetic host and osmoresistant transformants have been identified whose characterization is underway.

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Title of the project	Glycoshield: surface modulation of the fungal and host response using a genomic approach
Title of your presentation	Phenotypic and transcriptional responses to protein N-glycosylation deficiency in *Candida albicans*

In order to investigate the importance of N-glycan modification of proteins in morphogenetic and pathogenic processes in *C. albicans*, we have disrupted the *C. albicans ALG5* and *ALG9* involved in the N-glycosylation pathway in the ER. In *S cerevisiae*, *ScALG5* encodes a UDP-glucose:dolichyl-phosphate glucosyltransferase that is a transmembrane-bound enzyme of the ER. *ScALG9* is required for the addition of two different □-1,2-linked mannose residues to the lipid-linked oligosaccharide (LLO). We have also characterized the *Camnn9* null mutant defective in a □-1,6 mannosyltransferase that adds mannose residues to the outer chain of N-glycans in the Golgi.

We have demonstrated that the tree mutants *Caalg5*, *Caalg9* and *Camnn9* show several phenotypes consistent with the alterations of surface cell (For example, *Camnn9* is more sensitive to calcofluor white and congo red than the isogenic wild-type strain while *Caalg5* and *Caalg9* show an increase in resistance to these compounds) and that *Camnn9* null mutant is unable to grow as true hypha while the other null mutants do not show any alteration in the morphogenetic process. All the null mutant cells invade the agar surface more than the wild type under conditions that normally support growth by budding. Most notably, this phenotype is shown by heterozygous strain *alg5/ALG5* and *alg9/ALG9.* Moreover, deletions of *ALG9* and *MNN9* promote flocculation in minimal medium. The three mutants exhibit reduced Alcian blue binding (Approximately 90, 85 and 50% of the wild-type dye binding activity for *alg5*, *alg9* and *mnn9*, respectively). N-glycan chains are necessary for normal pathogenic host-fungus interaction because *Camnn9* mutant is highly attenuated in virulence. Deletion of the *ALG9* gene has a minor effect on virulence and the Ca*alg5* mutant behaves as the wild type strain. Because *Caalg9* shows a lower growth rate than wild-type and slight or not significant differences in the growth have been appreciated in *Camnn9* and *Caalg5,* respectively, we conclude that growth defects alone do not account for the virulence defects. In order to know the cellular localization of the Alg5 and Mnn9 proteins in *Candida albicans*, we have introduced into each mutant strains a plasmid that allows expression of the carboxy-terminal fluorescent fusion protein. Fluorescent is localized on citoplasmatic patches which probably correspond to endoplasmatic (Alg5p-GFP fusion) and Golgi membranes (Mnn9p-GFP fusion).

We have carried out the transcriptome of the three mutants. We have also analysed the response of *C. albicans* cells treated with inhibitor tunicamycin (at 20 □g/ml during 1 hour) that specifically blocks the first step in the synthesis of LLO. At least three independent replicates were performed for each experiment and genes that displayed statistically significant changes in expression level (> 1.5-fold) were identified. Tunicamycin induces expression of genes related to endocytosis, actin cytoskeleton and stress response associated to the protein translocation and represses biogenesis of cellular components. The mayor class of genes differentially expressed in the *alg5* null mutant overlapped significantly with that of a starvation response and included those required for glycolysis and gluconeogenesis, catabolic process and biosynthetic process. Genes required for normal function of mitochondria are down-regulated. In *alg9* null mutant genes related to osmotic and salt stress, proteomic degradation, actin cytoskeleton and cell adhesion are up-regulated. Over expression of ubiquitin pathway genes suggests that *ALG9* is required for the proper folding of proteins into ER. Several *ALS* genes are also up-regulated, this is consistent with phenotype of flocculation and invasive growth shown by this mutant. Loss of Mnn9p leads to alteration of several genes involved in filamentous MAP Kinase pathways suggesting that in exponential growth this pathway are less functional than in the wild-type. Between the common genes differentially expressed in the three mutants (19 up and 6 down-regulated) we found genes that encode for cell surface proteins like Adh5 and Pga62 (ScFlo1) and genes that are induced in stress response or have been associated with morphogenetic process. Interestingly, ten common genes are differentially over expressed in *alg5* and *alg9* null mutants and cells treated with tunicamycin. Four of them encode for cell surface proteins (Adh1, Pga62, Pir1, Gph1). Taken together our results suggest that N-glycosylation defects induce common stress responses that try to alleviate alterations in the cell surface.

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Title of the project	Glycoshield: Surface modulation of the fungal & host response using a genomic approach
Title of your presentation	Analysis of surface alterations in *Candida albicans* MAPK mutants by flow cytometry

Previous data from our group described the essential role that at least 3 MAP kinase pathways have in the stress response, morphogenesis and cell wall integrity in the fungal pathogen *Candida albicans*.

In this work we wondered whether recognition, internalization and host response mediated by immune cells could be altered in contact with different SAMs (Surface Altered Mutants) in *C. albicans*, specially those dealing with MAPK signaling elements. *hog1* mutants, which are sensitive to oxidative stress and avirulent in a murine model, were more efficiently killed by both PMNs and murine macrophages, while deletion of *CEK1* results in cells more resistant to phagocytes. No differences were found for *mkc1* mutants compared to the wild type. Cells lacking some of the elements of the Cek1-mediated pathway were more efficiently phagocytosed by murine macrophages and higher levels of TNF could be detected compared to those induced in contact to the wild type strain. We performed a comparative analysis of the cell wall surface in MAP kinase mutants by analyzing glycan expression on the fungal cell surface by flow cytometry by using specific antibodies against α and β 1-2 mannosides, quitin and β 1-3 glucan. Our results demonstrate that deletion of certain elements of the *CEK1*-mediated pathway (such as *CEK1* or *HST7*) express higher levels of glycans on the cell surface compared to the wild type strain. When mapping the different epitopes on the different families of cell wall mannoproteins (CWMP) released from cell wall we observed that CWMP mannosylation in both non covalent linked proteins (released by heat treatment) and GPI-linked proteins (released by zymolyase treatment) was different in *cek1* mutants. We also observed higher levels of β 1-3 glucan in the zymolyase fraction compared to the isogenic wild type strain. This altered cell wall present in *cek1* mutants was confirmed by visualizing the cell wall by TEM. Deletion of *CEK1* or *HST7* renders cells more susceptible to phagocytosis by different cell lines (HL-60 cell line, human neutrophiles and murine macrophages) and an enhanced binding which was partially blocked with lactose.

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Title of the project	Surface modulation of the fungal and Host response using a genomic approach
Title of your presentation	**Analysis of *Candida albicans* cell wall glycans during phagocytosis by macrophage**

Analysis of *Candida albicans* cell wall glycans during phagocytosis by macrophage

Aurore Sarazin, Maria Martínez-Esparza, Daniel Poulain and Thierry Jouault

Recognition of yeasts and their clearance by macrophage is based on components of the yeast cell wall, which are considered part of its virulence attributes. Depending of the availability of the different cell wall glycans, immune cells response is directed differently. In this work we exploit flow cytometry methods to probe and to follow up the availability of yeast surface glycans before and during phagocytosis by macrophages.

Our results showed that the expression levels of α- and β- linked mannosides as well as β-glucans can be successfully evaluated by flow cytometry using different specific antibodies. Exposition of different surface glycans of *C. albicans* and *S. cerevisiae* was evaluated by monoclonal or polyclonal antibodies directly or not coupled a fluorochrome for β- mannosides (5B2), α- mannosides (CA1 and Factor 1), β- glucans and chitine (β 1, 3- glucans and WgA). Presentation of surface glycans were shown to be differently available during phagocytosis by macrophages and varied depending on the strains (*C. albicans* or *S. cerevisiae)* and yeast live or heat-killed and on the yeast: macrophage ratio (1:1 or 10:1).

For *C. albicans*, exposition levels of β- mannosides as well as α- mannosides in proportion 10:1 increased during phagocytosis by macrophages while β- glucans and chitine were not available. For 1:1 ratio, exposition levels of β- mannosides and α- mannosides of *C. albicans* were decreased during phagocytosis whereas β- glucans and chitine were detected. For *S. cerevisiae*, presentation of surface glycans during phagocytosis is less shifting.
In contrast to live yeast cells, when using heat killed yeasts, no difference could be observed between 1:1 and 10:1 ratios whether for *C. albicans* or *S. cerevisiae*.

Together these results show that depending on the *C. albicans* burden, glycans are differently accessible to innate immune receptors during phagocytosis by macrophages. However when heat killed yeasts which are not able to modulate their cell wall components, were used, no difference could be observed suggesting that glycans availability during phagocytosis may result of a response of yeasts to the phagosome environment.

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Title of the project	Glycoshield
Title of your presentation	Structure and function of the signaling mucin Msb2 in *Candida albicans*

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The Msb2 protein of *Candida albicans* functions as an environmental sensor regulating morphogenesis, cell wall biogenesis and stress resistance. Msb2 is inserted into the cytoplasmic membrane by a single transmembrane region separating a large, highly glycosylated extracellular domain and a short intracellular domain. We constructed a *C. albicans* strain synthesizing a variant Msb2 protein, which carries an HA-tag in the extracellular domain and a V5-tag in its cytoplasmic portion. The functionality of the tagged Msb2 protein was verified by complementation of *msb2* mutant phenotypes. Results on deleted Msb2 variants demonstrated that the extracellular domain and the transmembrane region are required for full functionality of Msb2. Immunblotting experiments revealed that *MSB2* is highly expressed from its native or the heterologous *ACT1* promoter and that large quantities of Msb2 are secreted into the growth medium. Secreted Msb2 only contains the HA- but not the V5-tag. In contrast, the V5-tagged cytoplasmic domain of Msb2 was exclusively present in the cell extract. We conclude that Msb2 represents a signaling mucin, which is cleaved proteolytically into extracellular and cytoplasmic portions. Gelfiltration analysis revealed that the secreted Msb2 portion has a molecular mass of >500 kDa indicating that it is highly glycosylated. As shown by immunblotting experiments the protein *O*-mannosyl transferases Pmt1 and possibly Pmt2 appear mostly responsible for the glycosylation status of Msb2.

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Title of the project	HELDIVET
Title of your presentation	Genomics of non-Helicobacter pylori gastric Helicobacter species

Quasi-complete genome of canine gastric strains *H. bizzozeronii* CCUG 35545^T and *H. salomonis* CCUG 37845^T , and a recent human gastric *H. bizzozeronii* isolate CIII-1 (Kivisto et al 2009) have been obtained using the 454-pyrosequencing technique, SOLID sequencing (2008) and linker-PCR. Estimated sizes for these genomes are ~1.7 Mb. Several approaches have been tested to lengthen the sizes of contigs and make assembly of the genomes. The longest contig in *H. bizzozeronii,* CCUG 35545^T is 610733 bp and total number of contigs > 5000 bp is 24 (covering 1.76 Mb). The longest contig in *H. salomonis* is 88 084bp and the number of contigs > 5000 bp is 60. *H. bizzozeronii* CIII-1 has been sequenced to 27 fold coverage (genome size ~1.75-1.8 Mb) and the number of contigs > 1000bp is 79. Based on homology with CCUG 35545^T the number of CIII-1 contigs > 5000bp is reduced to 23 (covering 1.75 Mb).

In order to identify the potential repertoire of variation mediated by simple sequence repeats (SSRs) in *H. bizzozeronii*, al list of SSRs (between one and six), and a number of repeat unit iterations was compiled for each of the two *H. bizzozeronii* shotgun genome sequences using MsatFinder. A total of 149 and 155 repeats were found in *H. bizzozeronii* CCUG 35545^T and CIII-1 respectively, with a density of ~ 1,04 repeat nt/kb. Homopolymeric runs of single nucleotide were the most frequent repeats found in both shotgun sequences (35-45% of all repeats). A total of 40 genes were identified potentially as phase variable. As described in *H. pylori*, the functions encoded by the putative phase-variable genes included lipopolysaccharides (LPS) biosynthesis, cell-surface associated proteins, and DNA restriction/modification systems.

Further studies have focused on the role of phase-variation in the modulation of the expression of LPS in *H. bizzozeronii*. Three putative phase-variable genes were selected: putative beta 1,4 galactosyltransferase (homolog of HP0826 of *H. pylori* 26695), bifunctional alpha-2,3/-2,8-sialyltransferase (homolog of *C. jejuni cstIII*) and UDP-GlcNAc 2-epimerase (homolog of *C. jejuni neuC*). The sequences of the entire locus (~5kb) containing the *cstIII* and *neuC* homologues from six canine and two human *H. bizzozeronii* strains showed high diversity among different strains in the number of *cstIII* copies and in the distribution of mono SSRs in the locus.The LPS profile analysis of multiple *H. bizzozeronii* strains performed with and without neuraminidase treatment confirmed the presence of sialic acid in the LPS of canine strains. The LPS of both human strains were not sensitive to neuraminidase treatment indicating either the absence of sialic acid in LPS or different level of LPS sialylation compared to the canine strains.

Our future studies include to continue assembly of both *H. bizzozeronii* and *H. salomonis* type strains. In January 2010, Life Sciences 454 pyrosequencing technology will be used to acquire quasi-complete genome sequences of two additional human *H. bizzozeronii* isolates, belonging to the same clone, obtained from antrum biopsies of the Finnish patient, both before and after the therapy, with a time interval of ten months. These genomes will be aligned and used to analyse gains and/or losses of genes, and to estimate the recombination frequency by measuring the number of single nucleotide polymorphisms (SNP) and clusters of polymorphisms (MNP).

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Title of the project	*Pneumocystis* PathoGenoMics: Unravelling The Colonization-to-Disease Shift
Title of your presentation	Comparison of proteomic profiles in the Bronchoalveolar lavage fluid of Idiopathic pulmonary fibrosis patients with and without *Pneumocystis* colonization

Introduction

Since a quantitative proteomic approach with isobaric labelling (iTRAQ) has recently been suggested to be highly suitable for the study of differentially expressed proteins related with different clinical conditions, we have now used this method to examine for potential quantitative changes in the proteins from the bronchoalveolar lavage (BAL) fluid of patients with Idiopathic pulmonary fibrosis (IPF) colonized by *Pneumocystis jirovecii* in comparison to non-colonized IPF patients.

Methods

AL fluid from 10 well characterized IPF patients (5 colonized and 5 non-colonized by *P jirovecii*) were collected and centrifuged to 600xg during ten minutes and supernatant were pooled separately. Supernatant pooled were concentrated with Microcon Ultracel YM-3 (Millipore) and 200 ug of total proteins was Albumin and IgG depleted using the Depletion Spin Trap Kit of GE Healthcare. Resultant proteins were methanol:chloroform precipitated and following resuspended in 0.1 % SDS to proceed trypsin digest, ITRAQ Labelling and LC-MS/MS analysis.

Results

136 different proteins were detected in BAL fluid. High confidence sequence information and expression levels for 65 polypeptides, including metabolic enzymes, immunity-related proteins, surfactant associated protein, calcium-binding proteins, redox protein, peptidases and proteases were reported. Thirty-four proteins displayed higher levels of expression in colonized patients whereas twenty-eight were more expressed in non-colonized patients.

Conclusion

There are a different proteomic profiles in the BAL fluid of Idiopathic pulmonary fibrosis patients with and without *Pneumocystis* colonization. *P. jirovecii* colonization may play a role in the pathophysiology of Idiopathic pulmonary fibrosis through changes in proteins expression. However, future studies are needed to further define the role of *Pneumocystis* infection in Idiopathic pulmonary fibrosis.

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Title of the project	*Pneumocystis* PathoGenoMics: Unravelling The Colonization-to-Disease Shift
Title of your presentation	*Pneumocystis carinii* Cell and Biological Cycles: Ploidy and Trophic-to-Cystic Form Transition

The *Pneumocystis* pathogenic power appears clearly linked to the proliferation capacity of these fungi. However, *Pneumocystis* multiplication processes have been only rarely explored mainly because *Pneumocystis* organisms could not be continuously cultured. In fact, exploring the multiplication process of *Pneumocystis* organisms requires the examination of their life cycle as well as their cell cycle. Our strategy aims firstly at separating and purifying trophic and cystic forms of *Pneumocystis carinii* in order to approach differentiation and ploidy of life cycle parasite stages, and secondly at testing the infectious capacity of separated forms using either *in vitro* or *in vivo* models.

A reliable high speed sorting system (FACSAria cytometer, Becton Dickinson) was used. Following specific coimmuostaining of both trophic and cystic forms of *Pneumocystis*, host cell debris were successfully eliminated and highly pure trophic and cystic form populations were reproducibly separated and collected for further analyses with a purity of 99,6 to 100%. These sorted populations were shown to remain infectious in endotracheally-inoculated *Pneumocystis*-free Nude rats.

First, growth kinetics of either pure trophic or cystic forms of *P. carinii* was followed *in vitro* (i) in an axenic short-term culture model or (ii) in co-culture with rat epithelial alveolar cells (L2 cell line, ATCC CCL no.149). Second, either pure trophic or cystic populations were endo-tracheally inoculated to *Pneumocystis*-free Nude rats. The results of these experiments indicated that trophic forms can apparently multiply *in vitro* on their own but that they cannot develop into cystic forms, in contrast to what happens in the rat model. This observation may explain the absence of continuous growth of *Pneumocystis in vitro*. Third, DNA intercalating agent (Sytox® green dye, Invitrogen) allowed us to measure DNA contents of sorted trophic and cystic forms in comparison with haploid and diploid *Saccharomyces cerevisiae* reference strains.

To complete our study, expression profiles of sorted *Pneumocystis* populations are currently investigated using deep-sequencing (Genoscreen, Lille, France) and microarray approaches (University of Cincinnati, USA). All together, these results should shed new light on the intricate modes of multiplication of these opportunistic micromycetes.

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Title of the project	ERA-RNAi Net WP7
Title of your presentation	Investigation of host cell requirements for Yop injection by Yersinia into host cells (Poster presentation)

Yersinia outer proteins (Yops) are directly delivered into host cells via a type three secretion system. To study Yop injection into host cells, a reporter system was developed using a YopE-beta lactamase as a reporter to detect Yop injection in single cells. Using this reporter system we could demonstrate that binding to and signal transduction via □1 integrins is crucial for Yop injection into host cells. Pretreatment of cells with siRNA specific for different RhoGTPases revealed that both RhoA and Rac1 but not Cdc42 are crucial for Yop injection. Based on these findings we continued these studies using as well chemical inhibitors as well as a RNAi screening approach to define in more detail host factors involved in the signal transduction cascade required to facilitate Yop injection. Examples of first screening result will be discussed.

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Title of the project	ERA-RNAi Net WP8
Title of your presentation	Factors involved in Bartonella induced VEGF secretion

Bartonella henselae causes vasculoproliferative disorders in humans. Activation of hypoxia-inducible factor-1 (HIF-1), the key transcription factor involved in angiogenesis, was detected in *Bartonella henselae*-infected host cells in vitro and in vivo. Previous microarray analysis revealed that a *B henselae* infection resulted in the activation of genes typical for the cellular response to hypoxia. Hypoxia induced factor HIF-1a was essential for B. henselae-induced expression of vascular endothelial growth factor (VEGF) as shown by inhibition with the use of HIF-1-specific short-interfering RNA. Moreover, infection with B henselae resulted in increased oxygen consumption, cellular hypoxia, and decreased ATP levels in host cells. In similar it was shown that also other bacteria either due to oxygen consumption or due to the production of siderophore such as Salmonella or Yersinia induce due to HIF-1 □ activation many different HIF induced genes such as VEGF. To understand this host response in more detail we wanted to know whether other factors and if yes which factors beside HIF-1a are involved in regulation of VEGF secretion and whether there are differences found after infection for instance after infection with Bartonella or Yersinia. A first siRNA screen was established to test putative factors which might be involved in VEGF secretion. This studies revealed several factors especially transcription factors involved in basal secretion of VEGF, but only a few genes including HIF-1□ and HIF-2□ in Bartonella- and Yersinia- induced VEGF secretion. Some differences were found such as a significant requirement of p38 kinase and Akt3 for *Yersinia* but not for *Bartonella* induced VEGF secretion.

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Title of the project	RNAiNET WP04 - Identification of Host factors affecting immune activation by Klebsiella pneumoniae
Title of your presentation	Identification of Host factors affecting immune activation by Klebsiella pneumoniae

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We carried out a siRNA-screen targeting the human kinome to identify host factors modulating anti-inflammatory effects elicited by the pathogenic capsulated gram-negative bacterium Klebsiella pneumoniae.
We used an assay based on high-throughput automated fluorescence microscopy to detect the subcellular localization of a GFP-tagged p65 subunit of NF-kB, a key mediator of inflammatory signalling. With this assay we screened in lung epithelial carcinoma cells for reversal of a Klebsiella-induced block of IL-1b stimulated activation and translocation of p65/NF-kB.
Of ~640 pools (2 siRNAs/gene) we selected 36 targets for hit-validation with additional siRNAs based on Z-score analysis of 3 screening rounds. From this we considered 18 targets validated with at least 2 out of 4 siRNAs, and have begun the functional characterization for a select subset. Initial analyses suggest a role for ERK- and PKB/Akt-signalling in the Klebsiella pneumoniae-induced block of NF-kB activation.

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Title of the project	RNAi approach to unravel host cell functions modulating Yersinia enterocolitica infection
Title of your post	Development of Read-out system for RNAi screening of Yersinia-host interaction

Bacterial pathogens in the genus *Yersinia* are important human pathogens. *Y. enterocolitica* is food-borne and causes various gastrointestinal syndromes ranging from mild diarrhea to life-threatening septicemia. Pathogenesis of *Yersinia* is a complex process that is determined by a large number of virulence factors. Although considerable effort has been put into identification and functional characterization of various virulence factors, little is known about their regulation during *Yersinia* pathogenesis and even less of the involvement of host factors in the pathogenesis.

To employ high-throughput approaches such as RNAi screen for host factors involved in *Yersinia* infection and pathogenesis, we have been developing an bacterial side assay system. We first used a short half-life (40 min) GFP reporter pPROBEgfp(LVA) vector. Because of disturbing autofluorescence this was changed to a bacterial luciferase based reporter plasmid that we designed in-house. The new reporter plasmid allows us to detect changes in the response during the time-span of an RNAi-screening experiment.

We have cloned several known YeO3 virulence factor promoters into the pLux232oT vector, where the luciferase expression is under control of a bacterial promoter. Thus far we have analyzed the promoters of *yopE* (*PyopE*), the LPS O-antigen gene cluster operon 1 and 2 (*Pop1* and *Pop2*, respectively), and the outer core gene cluster (*Poc*), and activity of all these promoters were temperature-sensitive. *PyopE* is turned on at 37°C in the absence of $CaCl_2$; *Pop1* and *Poc* are expressed at higher level at room temperature. These pLux232oT reporters were introduced to different *Yersinia* hosts; *Y. enterocolitica* O:3 and O:8, *Y. pseudotuberculosis* O:1a and O:3, and *Y. pestis.* Testing the reporters with different cell types revealed that (i) *PyopE* reporter is nicely contact inducible with HeLa cells but not with AGS cell, (ii) contact-dependent induction with *Y. enterocolitica* was not as clear as that of *Y. pseudotuberculosis.* Therefore, further work will be continued with *Y. pseudotuberculosis* YPIII/pIB1, pLux232oT-PyopE reporter strain.

Based on the change of the reporter system, we are constructing a genomic random (promoter) library to plasmid pLux232oT, first in *E. coli* and, transformed the library into *Y. enterocolitica*. We expect that this library contains clones that carry constitutively active promoters and inducible promoters as well as clones without any promoter activity. For *E. coli* an estimation of 2500 promoters has been given. We are now screening the library at room temperature on LA plates for light expression. We will isolate clones that express light and save the non-expressing clones as a “black” library. We expect that the “black” library will carry additional promoters that are not active in bacteria grown on LA plates.

We have used the reporter strain *Y. pseudotuberculosis* YPIII/pIB1, pLux232oT-PyopE to optimise the conditions for tissue culture screening experiments using HeLa cell line.

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Title of the project	A global RNAi approach to unravel eukaryotic host functions that modulate bacterial infections
Title of your presentation	RNAi-based screening of fibroblast functions that restrain *Salmonella* intracellular replication

Salmonella enterica serovar Typhimurium is a Gram-negative bacterial pathogen that causes enterocolitis and systemic diseases in humans and animals. This pathogen interacts with different eukaryotic host cell types during the infection process. In vitro infection models have revealed that invasion of either macrophages or epithelial cells are followed by massive proliferation of the bacteria enclosed in vacuolar compartments. In marked contrast, *S.* Typhimurium persists inside fibroblasts and dendritic cells maintaining a non-proliferative state.

One of the tools used by this pathogen to manipulate host cell functions are dedicated type III secretion system (TTSS) that deliver bacterial effectors into the host cell. In this context, different host cell types may respond in distinct ways to these bacterial effectors. Our hypothesis contemplates the delivery of either *Salmonella* effector proteins(s) or other bacterial factors with capacity of signalling into the cytoplasm of fibroblasts. This process could lead to the activation of a host cellular activity that in turn would restrain the bacterial intracellular growth rate. To identify such host cell activities, we have investigated the role of kinases in this interplay by performing an screening of an siRNA library consisting of 1204 siRNAs (2 siRNAs per kinase).

The screening design is based on detecting bacteria expressing the fluorescent protein DsRed in human fibroblasts. siRNA-mediated knockdown of host cell protein(s) that limit Salmonella growth, resulted in the overgrowth of *Salmonella* inside the cell, what was detected by a net increase in the fluorescence signal. We identified several candidate hits, including kinases clustering in the PI3K-Akt functional network, which are currently in the validation phase using four different siRNAs per kinase. This design ensures robust and accurate identification of host cell factors limiting *Salmonella* intracellular replication. New kinases not previously assigned a role in the bacteria-fibroblast infection model will be further investigated individually at the functional level.

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Title of the project	SPATELIS
Title of your presentation	Characterization of new *Listeria monocytogenes* surface proteins of the LPXTG family

Abstract

Characterization of new *Listeria monocytogenes* surface proteins of the LPXTG family

Javier F. Mariscotti[1], Enrique Calvo[2], Laura Botello-Morte[1], M. Graciela Pucciarelli[3], and Franciso García-del Portillo[1]

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Listeria monocytogenes is a Gram-positive bacteria pathogen that causes listeriosis, a severe food-borne disease in humans. This bacterium has the ability of cross three host barriers during infection: the intestinal, the blood-brain, and the feto-placental barriers. *L. monocytogenes* also invades phagocytic and non-phagocytic host cell types in which it survives or proliferates. The genome of *L. monocytogenes* encodes 41 proteins that are anchored covalently to the peptidoglycan via the recognition by a sole enzyme, the sortase SrtA, of a conserved LPXTG motif located in the C-terminus. These surface proteins are candidates to play an important role in the host-pathogen interaction. Some of the few proteins of this family characterized at the functional level include the invasins Internalin-A (InlA), and Vip; together with InlJ, which promotes adhesion. The role of the rest of LPXTG proteins remains unknown.

The goal of our study was to characterize six LPXTG proteins of *Listeria monocytogenes* in the context of the current ERA-NET project named SPATELIS, which addresses the biology of the entire LPXTG protein family. We constructed six defective mutants in the following LPXTG surface proteins: Lmo0159, Lmo0160, Lmo550, Lmo0725, Lmo2178 and Lmo2179, which were further subjected to phenotypic analyses. These mutants did not exhibit differences in invasion and intracellular proliferation in human cultured epithelial cells JEG-3. Using specific antibodies, we detected two of these proteins, Lmo0159 and Lmo0160, in the cell wall of *L. monocytogenes* grown in brain-heart infusion (BHI) medium and in intracellular bacteria growing inside epithelial cells. We also analyzed the cell wall proteome in these mutants to asses putative differences in the content of other LPXTG proteins. No significant differences were found in the relative amount of other LPXTG proteins. Future goals include the virulence analysis of these mutants in BALB/c model and the identification of putative host ligands involved.

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Title of the project	SPATELIS
Title of your presentation	Targeting the Met pathway activated by the invasin Internalin B from *Listeria monocytogenes*

Activation of Met by its ligand HGF leads to cell proliferation and scattering. Remarkably, this receptor is exploited by the bacterium *Listeria monocytogenes*, whose virulence factor InlB interacts with Met and triggers a signaling machinery, which leads to the immediate uptake of the pathogen into the cell. The N-terminal half of InlB is sufficient to bind and activate Met. Although the interaction of the corresponding $InlB_{36-321}$ to Met is structurally solved, it still remains obscure which downstream signal components essentially regulate the process of invasion.

500 human protein kinases constitute the intrinsic part of the signal network in host cells. This study aims at the identification of human protein kinases that belong to the InlB/Met pathway. Recently, a quantitative phosphokinomics workflow was presented by us to reveal the transient phosphorylation of protein kinases in host cells activated by $InlB_{36-321}$. Among around 100 identified kinases, about 10 kinases were found to be regulated by $InlB_{36-321}$ including novel kinases, which were so far not be related to Met (Reinl et al., 2009). Interestingly, existing results of an ongoing HGF/Met study indicate significant signaling properties of $InlB_{36-321}$ in comparison to the physiological ligand.

In this project, full-length InlB ($InlB_{fl}$) (cooperation Dr. Niemann, Bielefeld), involving further co-receptors such as gC1q-R, Among aroundwill be used to analyze the host cell response. Therefore, my PhD project aims at the detailed analysis of signaling events at the protein kinase level in $InlB_{fl}$ stimulated cells to obtain an extensive overview of all kinases involved in bacterial uptake. This approach, for the first time, allows a direct comparison of Met stimulation by HGF and InlB to analyze the distinct function of InlB in mitogenic and motogenic processes as well as host cell invasion.

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Title of the project	ADHRESS
Title of your presentation	The "ADHRES Chip»: differential expression of *Pseudomonas aeruginosa* genes involved in ADHesion and RESistance to antibiotics.

Pseudomonas aeruginosa human infections are becoming serious health problems since these micro-organisms are mostly living as bacterial communities (biofilm) and are becoming multi-resistant to most existing antimicrobial treatments, these two properties leading to inefficient eradication. To understand which key genes are systematically expressed *in vivo* in *P. aeruginosa* biofilm-associated infections and that therefore could represent target genes for novel inhibitors, we first identified in available bacterial genomes, candidate genes involved in biofilm development (ADH). Up to now, twenty-five macromolecular systems that could play a role in adhesion have been highlighted and the corresponding loci present in multicopies, encode macromolecular systems that are probably acting in a synergistic way during the different stages of *P. aeruginosa* biofilm development. The definition of a sequential expression of *P. aeruginosa* genes (ADH signature) involved in biofilm development is therefore an important feature for characterization of the clinical situations in which *P. aeruginosa* forms biofilms and to know which types of biofilms (immature vs mature) are formed *in vivo*, therefore giving the possibility to provide a treatment “à la carte”.

We therefore engineered a dedicated microarray (Adhesive Chip or ADH-chip) that contains genes playing a role in *P. aeruginosa* biofilm. These genes encode structural elements of the different macromolecular systems : fimbriae assembled by chaperone-usher systems (CU), pili assembled by type IVa and IVb machineries, flagellum, exopolysaccharide (EPS) matrices (alginate, Pel, Psl, …), lectins, adhesive molecules secreted by type Va (autotransporters), type Vb (Two-Partner secretion systems) and type I secretion systems. It corresponds to 102 genes identified in the PAO1, PA14 and PAK strains. Using PCR, 88 genes were amplified from a *P. aeruginosa* PAO1 gene collection and 14 were PCR-amplified from genomic DNA derived from the other *P. aeruginosa* strains. We chose to add oligonucleotide tags to each PCR product that serves as an internal control for DNA spotting and for further normalization. PCR products together with serial dilutions of genomic DNAs were spotted onto nylon membranes with the spotter MicroGridII (Biorobotics) with 9 needles to create the *P. aeruginosa* ADH chips. Since this is a dedicated microarray (on which only genes participating to the same function have been spotted), normalization requires controlling the spotting prior to complex hybridization (CH). To do so, ADH microarrays are systematically submitted to a vector hydribization (VH) using a mixture of oligonucleotides complementary to tags present on PCR products and of 60 mer random oligonucleotides, both being radiolabelled. cDNA from *P. aeruginosa* were further hybridized and the signals obtained from CH have been normalized with signals obtained from the VH for each ADH microarray. This approach has been successfully validated by determining biofilm gene expression in Δ*retS* and Δ*ladS* strains, for which we know differential expression of ADH genes in comparison to their wild-type counterpart. The ADH-chip is currently validated on a collection of clinical strains isolated from chronically infected CF patients all along their disease and conserved in the laboratory.

We next plan to purify RNA directly from crude infected samples, thus limiting isolation step procedures that could distort the *in vivo* expression of biofilm gene expression. Moreover, as biofilms represent environmental conditions which could regulate antimicrobial resistance mechanism-associated genes, the ADH-chip will be updated and implemented with genes involved in antimicrobial resistance (RES), thus leading to the ADHRES-chip. Finally, this ADHRES-chip will not be limited to *P. aeruginosa* but will be enlarged to *Pseudomonas putida* and the *Burkholderia cepacia* complex. This tool is developed for infected human samples with the aim to help clinicians to adapt antimicrobial treatment and for fast and reliable diagnosis and prognostics.

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Title of the project	Identification of hot spots of divergence and rapidly changing genes within Shiga-toxin-producing Escherichia coli
Title of your presentation	Regulatory effect of H-NS in *Escherichia coli* ribonucleotide reductase genes

Ribonucleotide reductase (RNR) is an essential enzyme responsible of catalyzing the reduction of ribonucleotides to the corresponding deoxyribonucleotides and thereby provides the building blocks for DNA synthesis and repair. RNR is an indispensable enzyme for the DNA replication in all living cells. To date, in bacteria have been described three different classes of RNR (I, II and III) attending on its structure, iron ligand, radical and cofactor, but all of them have in common a tight allosteric regulation and the requirement of a protean radical to initialise the reduction. *Escherichia coli* have the particularity to encode in its genome for two subclasses of aerobic RNR; class Ia (*nrdAB*) and Ib (*nrdHIEF*) and one anaerobic RNR; class III (*nrdDG*).

H-NS is a nucleoide-associated protein (NAPs) that is involve in genomic compaction and also acts as a global regulator of gene expression in enteric bacteria in a response of environmental changes such osmolarity, pH or temperature. Normally this protein acts as a repressor and importantly H-NS is capable to make heteromeric interaction with other regulatory protein to modulate gene expression.

In this preliminary study we have demonstrated the effect of H-NS in the transcriptional regulation of *E. coli nrd* genes and its physical interaction to their promoter regions.