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# CUSO Doctoral Program in Microbial Sciences

# Prodoc Infection and Immunity, Lausanne, Geneva

## Annual Retreat 2011

## Villars, 8-9 September 2011

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# Program at a Glance

| Thursday | 8th September | 2011 |
|---|---|---|
| 12 : 00 - 13 : 00 | Welcome Sandwiches | |
| 13 : 00 - 13 :10 | Welcome address | |
| 13 : 10 - 13 : 50 | *Plenary talk*<br>Josep Casadesus<br>University Sevilla, Spain | *Chairs:*<br>Diego Gonzalez<br>Carmen Fernandez |
| 13 : 50 - 15 : 30 | *5 selected short talks*:<br>Elena Buttazzoni<br>Carmen Fernandez<br>Coralie Fumeaux<br>Peter Kupferschmied<br>Patrick Seitz | *Chairs:*<br>Diego Gonzalez<br>Carmen Fernandez |
| 15 : 30 - 16 : 00 | Coffee break | |
| 16 : 00 - 18 : 00 | Poster session | |
| 19 :00 - 21 :00 | Dinner | |

| Friday | 9th September | 2011 |
|---|---|---|
| 7 : 30 - 8 : 50 | Breakfast | |
| 8 : 50 - 9 : 30 | *Plenary talk*<br>Silke Stertz<br>University Zurich,Switzerland | *Chairs:*<br>Patrick Seitz<br>Giulia Pasqual |
| 9 : 30 - 10 : 00 | Coffee break | |
| 10 : 00 - 11 : 40 | *5 selected short talks*<br>Giulia Pasqual<br>Christelle Pythoud<br>Diego Gonzalez<br>Andrea Lohberger<br>Aurélie Gueho | *Chairs:*<br>Patrick Seitz<br>Giulia Pasqual |
| 12 : 00 - 13 : 00 | Lunch | |
| 13 : 00 - 15 : 30 | Free activity | |
| 15 : 30 - 16 : 00 | Coffee break | |
| 16 : 00 - 16 : 40 | *Plenary talk*<br>Markus Meissner,<br>University of Glasgow, UK | *Chair:*<br>Aurelie Gueho |
| 16 :40-17 :00 | Prize Poster<br>Prize Talk<br>Farewell address | |

**Molecular characterization of the proteolytic processing of arenavirus envelope glycoprotein precursors by the cellular pro-protein convertase SKI-1/S1P**

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A crucial step in the life cycle of arenaviruses is the biosynthesis of the mature fusion-active viral envelope glycoprotein (GP) that is essential for virus-host cell attachment and entry. Here we characterized the molecular mechanism of the processing of the GP precursors (GPC) of the prototypic arenavirus lymphocytic choriomeningitis virus (LCMV) and Lassa virus (LASV) by the cellular proprotein convertase SKI-1/S1P. Our structure-function analysis revealed that the ability to process arenavirus GPCs, but not cellular substrates, critically depended on auto-processing of SKI-1/S1P at the B/B' recognitions site of its prodomain. Deletion mutagenesis further revealed that the transmembrane and cytoplasmic domains of SKI-1/S1P are dispensable for arenavirus GPC processing. Expression of a soluble form of the protease in SKI-I/S1P-deficient cells resulted in efficient processing of arenavirus GPCs and rescued productive virus infection. Interestingly, over-expression of a transmembrane stump generated by spontaneous shedding of SKI-1/S1P in the host cell affected SKI-1/S1P processing of cellular substrates, but not arenavirus GPCs. These remarkable differences are the first evidence for a differential mechanism of molecular recognition underlying SKI-1/S1P processing of viral GPCs and cellular substrates. Despite efficient GPC processing upon expression of soluble SKI-1/S1P excess exogenous soluble SKI-1/S1P was unable to process arenavirus GPC at the surface of SKI-I/S1P-deficient cells.

**Analysis of the DEAD-box RNA helicase SA1387 from *Staphylococcus aureus***

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DEAD-box RNA helicases are a large family of RNA helicases and are found in almost all organisms. They are characterized by the presence of nine conserved motifs that are necessary for ATP hydrolysis and RNA binding. The members of this family show a RNA-dependent ATPase activity. *In fine*, the energy from the hydrolysis can dissociate RNA duplexes or RNA-protein complexes.

In bacteria, RNA helicases were shown to be required for ribosome biogenesis and are often induced for growth at low temperature and other stress conditions.

SA1387 codes for a DEAD-box helicase in *Staphyloccoccus aureus* and its role is still unknown. It is found in an operon together with SA1386, a type IV endonuclease, and both genes exhibit homology to a similar operon in *Bacillus subtilis*, in which the two proteins have been characterized. Despite large efforts to detect a biochemical activity the helicase displays no *in vitro* activity. This could be explained by the absence of a protein partner or a specific substrate as it has been found for others DEAD-box helicases.

We have made SA1387 and SA1386 single mutants using the TargeTron™ Gene Knockout System (Sigma-Aldrich) and, as expected, the SA1387 mutant displays a cold sensitive phenotype, which is rescued by complementation. For the endonuclease there is no phenotype, however, a double mutant SA1386-SA1387 is not viable, which suggests a genetic (functional?) interaction between the RNA helicase and the DNA endonuclease.

The further aims of this work, is to better characterize SA1387 and to understand the interaction between these proteins.

**Regulation of the activity of DnaA and its role during the initiation of chromosomal replication in *Caulobacter crescentus***

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DnaA binds to the *Caulobacter crescentus* chromosomal origin and initiates DNA replication only once per cell cycle. We previously discovered an essential protein in *C. crescentus*, HdaA, whose depletion in the cell leads to over-replication and filamentation. HdaA co-localizes with DnaN, the β-clamp of the DNA polymerase III, throughout replication. Its homologous counterpart in *Escherichia coli* (Hda) inactivates DnaA by stimulating its ATPase activity.

We generated point mutations in two conserved domains of HdaA and each inactivated the HdaA protein. We showed that the first domain, at the N-terminus, was required for the *in vivo* interaction of HdaA with DnaN, and the subsequent recruitment of HdaA to the DNA polymerase complex. The second domain is homologous to the AAA+ domain of DnaA and it was not involved in the HdaA-DnaN interaction or in the colocalization of HdaA with the DNA polymerase complex. Instead, this domain may be involved in an interaction between HdaA and DnaA, to stimulate the inactivation of DnaA, a hypothesis that we are currently testing.

We also mutated the Arg finger in the DnaA AAA+ domain. We observed that the expression of the resulting mutant DnaA protein caused severe over-initiation of chromosomal replication that blocked cell division, unlike the expression of the wild-type DnaA at similar levels.

We propose that HdaA inactivates DnaA once the DNA polymerase is replicating the chromosome and that this mechanism is dependent on an interaction between the N-terminus of HdaA and DnaN and on two conserved Arg fingers in DnaA and HdaA.

**Regulation of unusual developmental phenotypes by the MucRs transcription factors in *Caulobacter***

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*Caulobacter crescentus* undergoes a complex life cycle, during which the flagellated swarmer cell differentiates into an adhesive stalked cell, its replicative form. At the end of the division, a stalked and a swarmer daughter cell that carries newly synthesized flagellum and pili are produced. Biosynthesis of the pili occurred only in the late stage of division, as soon as the pilus subunit, pilin encoded by *pilA*, is expressed. It was assumed that the level of phosphorylated CtrA was responsible for the delay in PilA expression.

We suggested that this delay was caused by a negative regulator probably controlled by the PleC histidine kinase/phosphatase. *pleC* deletion presents many defects, including pili biogenesis. The absence of pili was thought to be in part due to the low level of phosphorylated CtrA.

We show that reduced CtrA activity is not responsible for the *pilA* expression defect in a *pleC* mutant. Instead, the *pleC* mutant seems unable to relieve repression by two newly identified orthologous pair of repressors, MucR1 and MucR2. By screening for mini-Tn*5* insertions that restore expression of a $P_{pilA}$-*nptII* transcriptional reporter construct in *pleC* mutant cells, we identified a gain-of-function mutation in an uncharacterized gene, *mucR1* that shares 70% homology with Ros-MucR transcriptional regulators of exopolysaccharide production in alpha-proteobacteria. As a homolog of MucR1 (MucR2) is also encoded in the *C. crescentus* genome, we inactivated both mucR genes and found that the *mucR1/2* double mutation restored *pilA* expression to the *pleC* mutant cells. Moreover, loss of both MucR`s caused morphological and developmental defects, including the loss of motility and cell density changes. We identified transposon insertions that restore both phenotypes and are currently studying the regulatory mechanism that cause suppression.

**Nanoparticles as antigen carriers: a new tool to fight arenaviruses**

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Lassa virus (LASV) causes a severe hemorrhagic fever with high mortality, and represents a serious public health problem. Considering the number of people affected and the restricted therapeutic options, a safe and efficacious LASV vaccine is urgently needed. Therefore, we aimed to develop a novel recombinant LASV vaccine based on a polypropylene sulfide (PPS) nanoparticles (NPs) platform and its evaluation in a small animal model.

During the first phase of the project, we designed and produced suitable vaccine antigens (Machupo glycoprotein 1 known to be a potent inducer of the humoral response and lymphocytic choriomeningitis virus (LCMV) peptides corresponding to CD8 T cells epitopes that were nanoparticles-coupled and subcutaneously delivered to mice to evaluate the ability of our antigen-conjugated NPs to elicit an anti-viral antibody and T cell response *in vivo*.

Detection of specific antibodies against MACV GP1 in ELISA revealed a strong and specific antibody response against MACV GP1 in mice immunized with MACV GP1-conjugated NPs.

High frequencies of LCMV antigen specific CD8 T cells were also measured after mice immunization with NPs-coupled LCMV peptides but we still not know if they are protective.

**In *Caulobacter crescentus,* the artificial production of the alarmone (p)ppGpp retards cell differenciation and initiation of chromosome replication**

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In *Escherichia coli,* starvation triggers, via the alarmone (p)ppGpp, an adaptive arrest of the cell division, but little is known about how more complex bacterial cell cycles respond to such conditions. *Caulobacter crescentus* has a two-stage life history, presenting a juvenile motile form, the *swarmer cell* that has to differentiate into a reproductive sessile form, the *stalked cell*, before replicating its DNA and dividing asymmetrically. The genome of *C. crescentus* encodes a (p)ppGpp synthetase, that is required to delay the initiation of DNA replication under conditions of carbon starvation. By contrast, the influence of (p)ppGpp accumulation on the differentiation process has not been convincingly assessed yet.

Here we report the construction of a *C. crescentus* strain in which a stringent response can be induced in the absence of actual starvation by the expression of the gene *relA',* coding for a constitutively active form of RelA, a (p)ppGpp synthetase from *E. coli*. By studying synchronized populations, we observed that the expression of RelA' is sufficient to significantly retard the replisome assembly and the initiation of DNA replication, most likely by stimulating the proteolysis of DnaA. Furthermore, using fluorescently labeled proteins that localize at different stages of the cell cycle, we could demonstrate that important molecular events characteristic of the swarmer-to-stalked cell transition are delayed as well when RelA' is induced. We propose that, in stringent conditions, the intracellular accumulation of (p)ppGpp could favor dispersal and foraging functions over multiplication by extending the lifespan of the motile, non-reproductive swarmer cell, and thus play a major role in the ecological adaptation of *C. crescentus* to nutritional stresses.

**Characterisation of *Mycobacterium marinum* niches**

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Tuberculosis remains a world-wide health issue. *Mycobacterium tuberculosis*, the agent responsible for this disease, is able to manipulate the phagocytes of the innate immune system. After uptake by phagocytosis, it stops the maturation of the phagosome where it resides and establishes a niche where it can proliferate. Our aim is to characterize the virulence mechanisms and the manipulation of the phagocytic pathway. For that, we make use of the soil amoeba *Dictyostelium* as a host for a pathogenic mycobacterium, *M. marinum*, a close cousin of *M. tuberculosis*. *Dictyostelium* normally uses phagocytosis for feeding purposes and its phagocytic pathway is very similar to the macrophage one. *M. marinum* infects the amoeba and arrest phagosome maturation as it does in macrophages.

We established a protocole to isolate pure fractions of compartments containing the pathogenic strain *M. marinum* or the non-pathogenic strain *M. smegmatis* or the avirulent strain *M. marinum*-L1D. Preliminary characterization of isolated niches at 1 hour post infection by immunofluorescence and western blotting shows the presence of both early (VatA) and late endosomal markers (p80, LmpA), of mitochondrial markers (mitochondrial porin) and endoplasmic reticulum markers (calreticulin, PDI). Then, using TMT isobaric labeling and mass spectrometry, we compare the proteomic composition of those isolated compartments to determine the impact of pathogen manipulation to divert the bactericidal phagosome into a friendly replication niche. This will allow us to identify host factors that modulate resistance or susceptibility to infection and mycobacterial proteins expressed intraphagosomally and potentially involved in the manipulation of phagosome maturation.

**When *Pseudomonas fluorescens* CHA0 launches its insect toxin production**

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*Pseudomonas fluorescens* are well-known disease suppressive bacteria in the rhizosphere of various plant species. The plant-beneficial *P. fluorescens* strain CHA0 produces many secondary metabolites which protect the plant roots against fungi and was discovered to also exhibit potent systemic and oral insecticidal activity. The toxicity towards insects relies on the production of a novel large protein toxin termed Fit and additional yet unidentified bacterial factors. The Fit toxin is part of a virulence cassette coding for regulators and a type I secretion system predicted to function in toxin export.

By using various techniques of molecular biology, such as fluorescence microscopy and quantitative real-time PCR, we aim at identifying mechanisms and signals that control the biosynthesis, secretion and biological activity of the insect toxin and accessory virulence factors, and at understanding their ecological role in the plant root environment.

We recently visualized the induced toxin expression of CHA0 during a systemic infection of insect larvae, while toxin production in the wild type strain was not observed on healthy plant roots and in common culture media. Studies of fluorescent reporter strains in a distinct growth medium mimicking some of the conditions found in insect haemolymph revealed interesting insights into the molecular basis of the induction of the Fit toxin expression within insects.

**Natural competence of *Vibrio Cholerae* is subject to three regulatory pathways**

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The human pathogen *Vibrio cholerae* is a normal member of aquatic habitats. Within this environment *V. cholerae* colonizes the chitinous exoskeleton of small zooplankton. Chitin is a polymer of N-acetylglucosamine that, apart from being a nutrient source, induces the developmental program of natural competence for transformation in this organism. Natural competence for transformation is a mechanism of horizontal gene transfer consisting in the uptake of free DNA from the environment followed by its integration into the genome of the acceptor strain. In *V. cholerae* natural competence is not only regulated by the inducer chitin but is also subjected to the regulation by quorum sensing (QS) and carbon catabolite repression (CCR). The aim of this study was to investigate whether the competence genes are uniformly regulated by chitin, QS and CCR. We created transcriptional reporter fusions between the promoter regions of three representative competence genes and genes encoding fluorescent proteins. The reporters were tested under different growth conditions: chitin surfaces that mimic *V. cholerae* growth on chitinous exoskeleton of zooplankton, and chitohexaose-containing liquid medium, where chitin availability is homogeneous. The reporters were also tested in *V. cholerae* strains, which were defective in intermediates of the QS and CCR pathways. Based on these results we conclude that not all competence genes are regulated in the same manner. Furthermore efficient transformation only occurs if all three regulatory pathways, chitin, QS and CCR, are intact. These findings highlight once more the complex regulatory circuit of chitin-induced natural competence in *V. cholerae*.

**The deletion and hyperactivity of the transcription factor *UPC2* involved in azole resistance impacts on virulence of *Candida albicans.***

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As a cause of resistance to azoles several mechanisms are reported, among them overexpression of *ERG11*, encoding Erg11p (P450 cytochrome lanosterol 14α-demethylase), which is involved in the biosynthesis of ergosterol and is the target of azole antifungal drugs. *ERG11* is positively regulated by the zinc finger transcription factor *UPC2*. Several activating point mutations (gain-of-function, GOF mutations) in *UPC2* are known leading to overexpression of *ERG11*.

In this study we addressed the role of the known GOF G648D in the transcription factor Upc2p on *C. albicans* virulence by measuring mice survival and kidneys colonization by counting fungal tissue burden in a *C. albicans* infection model. Therefore, a set of strains was designed in which the ORF of the transcription factor was deleted for both alleles in the wild type strain SC5314. Next the entire ORF of either the same wild-type allele or a GOF-containing allele were re-introduced at their genomic loci.

Our results show that the *UPC2* deletion mutant (DSY4357) and the revertant strain containing the *UPC2* allele with the GOF-mutation (ALY18) showed significant decreased virulence as compared to the wild-type strain SC5314. In contrast, the kidney tissues burden showed only significant decreased fungal burden for the deletion strain DSY4357 in comparison with the wild-type strain SC5314 but not for the strain containing the GOF mutation ALY18. These results suggest that decreased virulence is not always correlated with a corresponding decrease in tissue burdens. In conclusion, the development of azole resistance by *ERG11* upregulation compromises *C. albicans* fitness in animal models.

**PCSK9 is a novel cellular factor against arenavirus infection**

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BACKGROUND

Arenaviruses are the causative agents of severe hemorrhagic fevers in humans in Africa and South America. The cellular receptor for Old World arenaviruses, including the prototypic arenavirus lymphocytic choriomeningitis virus (LCMV) and the highly pathogenic Lassa fever virus (LASV), is dystroglycan (DG), a cell surface receptor for proteins of the extracellular matrix. LASV and LCMV use an unusual pathway for cell entry that is independent of clathrin, caveolin, dynamin and actin and fuse in late endosomal compartments.

Proprotein Convertase Subtilisin Kexin type 9 (PCSK9) is the latest discovered member of the Proprotein Convertases (PCs) family and was shown to play a crucial role in cholesterol homeostasis *via* the degradation of Low Density Lipoprotein receptor (LDLR). Interestingly, also the EH domain binding protein-1 (EHBP1), which plays important roles in protein recycling in endosomal structures, is PCSK9 dependent. Recently, PCSK9 was found to have a protective effect against hepatitis C virus (HCV) infection at the entry level, down-regulating LDLR and CD81, major HCV receptors.

AIMS

Investigate the protective effect of PCSK9 against LCMV infection *in vitro* and eventually identify PCSK9-dependent cellular factors involved in arenavirus life-cycle.

RESULTS

We found that HepG2 cells stably over-expressing PCSK9 are less susceptible to LCMV infection in contrast to HepG2 cells knock-down for PCSK9 that show the opposite phenotype. The non related vesicular stomatitis virus (VSV), previously reported to be unaffected by PCSK9, was used as negative control. Accordingly, exposure to PCSK9

conditioned media or purified hPCSK9 partially protects all tested cell lines from LCMV infection (10µg/ ml purified PCSK9 induces ~50% infection reduction). Since this effect is also observed using MLV particles pseudotyped with LCMV or LASV GPCs, we hypothesize that PCSK9 interferes with arenaviruses at entry step. The mechanism of PCSK9 action is currently under investigation although our studies were able to exclude functional DG down-regulation by the proprotein convertase.

**Exploring the regulatory role(s) of metabolic fluctuations during the *Caulobacter crescentus* cell cycle.**

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Due to its particularity, *Caulobacter Crescentus* is a reference to study asymmetric cell division of prokaryotic organisms. Two different cells compound its population:

- The swarmer cell, a mobile cell containing a flagellum at one pole. Replication and division incompetent, maintained in a G1-like state of the cell cycle.
- The stalked cell, a sessile cell containing a cytoplasm elongation: the stalk, at the "old" cell pole. It can replicate DNA to allow cellular division.

The key event in *Caulobacter's* development is the transition from swarmer to stalked cell. This is managed by a phospho-relay-signalling pathway (Paul *et al.* 2008).

Our team identified KidO, an oxidoreductase homolog, as a bifunctional regulator of *Caulobacter's* cell cycle (Radhakrishnan *et al.* 2010). KidO indirectly stimulates DivJ kinase, a regulator of the circuitry controlling *Caulobacter* development. KidO also directly acts on the cytokinetic tubulin FtsZ, which assemble into the medial Z-ring, driving cytokinesis during the cell cycle.

Our study is now focusing on the first function of KidO, the cell cycle regulation. Using genome-wide approaches like ChIP deep Seq we want to identify targets of KidO implicated in cell cycle control.

References:

Paul, R., T. Jaeger, S. Abel, I. Wiederkehr, M. Folcher, E. G. Biondi, M. T. Laub and U. Jenal (2008). "Allosteric regulation of histidine kinases by their cognate response regulator determines cell fate." Cell **133**(3): 452-61.

Radhakrishnan, S. K., S. Pritchard and P. H. Viollier (2010). "Coupling prokaryotic cell fate and division control with a bifunctional and oscillating oxidoreductase homolog." Dev Cell **18**(1): 90-101.

**Old World arenaviruses enter the host cell via the multivesicular body and depend on the endosomal sorting complex required for transport**

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The highly pathogenic Old World arenavirus Lassa virus (LASV) and the prototypic arenavirus lymphocytic choriomeningitis virus (LCMV) use α-dystroglycan as a cellular receptor and enter the host cell by an unusual endocytotic pathway independent of clathrin, caveolin, dynamin, and actin. Upon internalization, the viruses are delivered to acidified endosomes in a Rab5-independent manner bypassing classical routes of incoming vesicular trafficking. Here we sought to identify cellular factors involved in the unusual and largely unknown entry pathway of LASV and LCMV. Cell entry of LASV and LCMV required microtubular transport to late endosomes, consistent with the low fusion pH of the viral envelope glycoproteins. Productive infection with recombinant LCMV expressing LASV envelope glycoprotein (rLCMV-LASVGP) and LCMV depended on phosphatidyl inositol 3-kinase (PI3K) as well as lysobisphosphatidic acid (LBPA), an unusual phospholipid that is involved in the formation of intraluminal vesicles (ILV) of the multivesicular body (MVB) of the late endosome. We provide evidence for a role of the endosomal sorting complex required for transport (ESCRT) in LASV and LCMV cell entry, in particular the ESCRT components Hrs, Tsg101, Vps22, and Vps24, as well as the ESCRT-associated ATPase Vps4 involved in fission of ILV. Productive infection with rLCMV-LASVGP and LCMV also critically depended on the ESCRT-associated protein Alix, which is implicated in membrane dynamics of the MVB/late endosomes. Our study identifies crucial cellular factors implicated in Old World arenavirus cell entry and indicates that LASV and LCMV invade the host cell passing via the MVB/late endosome.

**Subversion of the host cell's innate immune defense by human pathogenic arenaviruses**

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A hallmark of fatal arenavirus infections in humans is the inability of the patient's innate immune system to contain the virus resulting in uncontrolled infection, shock and death. The nucleoprotein (NP) of the prototypic arenavirus lymphocytic choriomenengitis virus (LCMV) was shown to counteract the host type I interferon (IFN) response by inhibiting activation of the IFN regulatory factor 3 (IRF3). In this study we show that the NPs of different arenaviruses (LCMV, Lassa virus, Junin virus, and Tacaribe virus) block signaling via the RNA-sensing retinoic acid-inducible gene (RIG-I) helicase. Arenavirus NPs intercept signal transduction leading to IRF3 activation downstream of the mitochondrial-bound CARD protein Cardif (IPS-1/MAVS/VISA). Using a co-immunoprecipitation approach, our experiments show that arenavirus NPs interact with one of the kinases involved in RIG-I/Cardif-mediated activation of IRF3, IKKε, but not with the closely related kinase TBK1. The interaction of NP with IKKε may interfere with the phosphorylation of IRF3, which is an essential step in IRF3 activation and type-I IFN induction. Moreover, the specific interaction with IKKε, but not TBK1, is striking and suggests selective perturbation of some, but not other pathways involved in innate anti-viral defense by arenaviruses.

**Unraveling the mechanism of DNA uptake in naturally competent *Vibrio cholerae***

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Natural competence is a physiological state of bacteria in which they are able to take up free DNA from the environment in order to integrate it into their genomes. *Vibrio cholerae*, a gram-negative bacterium causing the human disease cholera, has been shown to enter the competence state when growing on chitin surfaces within its natural aquatic habitat. To date, little is known about the actual mechanism of the DNA uptake process. A previous study has shown that three genes predicted to encode components of a type-IV pilus and a putative periplasmic DNA binding protein, are essential for natural transformation. All of these genes have closely related homologs in other bacteria. This prompted us to search for more *V. cholerae* competence genes by comparison to published data from other organisms. We identified a set of additional proteins that are potentially involved in the uptake or recombination of DNA in *V. cholerae*. By generating knockout strains of the respective genes and scoring their natural transformability, we were able to identify a number of components of a putative DNA uptake machinery. Furthermore, we developed a PCR-based assay that allows functional grouping of these components. This assay also allowed us to test whether DNA uptake is species-specific - as for other gram-negative bacteria such as *Neisseria gonorrhoeae* - or rather promiscuous. Taken together, these results enable us to present a first working-model for DNA uptake in naturally competent *V. cholerae*.

**Identification and characterization of novel antitubercular compounds**

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Over one third of the world population is infected by *Mycobacterium tuberculosis*, resulting in 2 million deaths and 8 million newly infected people every year. In addition, the efficiency of the existing therapies is threatened by the dramatic increase of multi-drug resistant strains. Many drug candidates identified by in vitro screens on *M. tuberculosis* fail when tested in vivo systems. Therefore, novel strategies, including phenotypic screenings directly in a host-pathogen system, are needed to discover antibacterial activities with high in vivo potency.

*Dictyostelium discoideum* allows performing convenient drug and genetic screenings. We developed high throughput assays to monitor the impact of chemicals and intracellular factors on bacteria health, growth, and virulence mechanisms as well as on intrinsic host defenses. We used mCherry and GF fluorescent reporters for *M. marinum* and *Dictyostelium* respectively. We validated the system using known antimycobacterial first and second line drugs, including isoniazid, rifampicin pyrazinamide, ethambutol, streptomycin, amikacin, kanamycin, and others, confirming the fluorescent measurements with visual inspection. Acanthoemoeba *castellani* was tested as an alternative host system. It allows experiments at higher temperatures, up to 37 ºC.

We are about to perform primary set of screening with the first 1000 compounds. In parallel host-pathogen system will be monitored by real-time PCR, visual inspection and will be confirmed by quantitative FACS assay. All positive hits will lead to establishing sets of derivatives. The place and mode of action of the most efficient ones will be investigated.

**Combinatorial screening for selective Hsp90 inhibitors against parasites**

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Unicellular parasites such as *Plasmodium, Leishmania* and *Trypanosoma* species reek havoc in countries of the developing world. It has been shown that their life cycles can be efficiently interrupted with Hsp90 inhibitors. We are developing live and *in silico* screening tools to find inhibitors that are selective or even specific for the pathogen Hsp90s. Our *in vivo* system consists of a panel of isogenic budding yeast strains, whose own Hsp90s are replaced with pathogen Hsp90 or human Hsp90α or Hsp90β. We had previously established, as a proof of concept, that pathogen and human Hsp90s can confer markedly different responses to established Hsp90 inhibitors in this setting. While setting up for high-throughput screens, we are already using this system to test new candidate compounds. In parallel, we are performing a variety of *in silico* virtual screens to identify novel drug candidates. In this context, we tried to maximize the rationality in comparing the inter-species differences in Hsp90's drug binding domain and adapted the screening protocol to select for the candidates that can interact with specific residues in *Plasmodium* falciparum Hsp90. With a proper rational drug design and the combinatorial approach to screen and validate hits, we expect to deliver the inhibitors that present favorable binding affinity to PfHsp90 compared with its human homologues. And hopefully, with a sufficient therapeutic window, these inhibitors might well find their way into the clinic to treat these deadly diseases.

**Exploring the roles of NADPH oxidases (NOX) in an amoeba model**

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Reactive oxygen species (ROS) are oxygen-derived small molecules that are highly reactive due to the presence of unpaired valence shell electrons. The membrane-bound NADPH oxidases (NOX) are considered as the major enzyme complexes devoted to ROS generation. In neutrophils and macrophages, NOX2 generated ROS are essential for the oxygen-dependent microbe killing mechanisms. The observation of severe infections in chronic granulomatous disease (CGD) patients is due to the deficient ROS generation in neutrophils and macrophages, which are deficient in NOX2 activity

In recent years, the social amoeba, *Dictyostelium discoideum*, has become a powerful and popular model to study cell intrinsic mechanisms of the innate immune response. By using the most sensitive ROS-detection methods now available, Amplex UltraRed (AUR), Dihydroethidium (DHE), and L-012, we were able to detect unambiguously the extracellular hydrogen peroxide and intracellular superoxide production in Dictyostelium. Our preliminary data indicated that various bacteria culture supernatants had significantly different stimulatory activities on ROS production. Further ongoing work demonstrates that structurally different lipopolysaccharide (LPS) and other potential TLR and NLR agonists can stimulate ROS production to extremely different levels. The use of a catalase (CatA) knockout Dictyostelium strain considerably increased the measurable level of extracellular H2O2, whereas inhibitors of the superoxide dismutase increased the measurable level of intracellular superoxide. In contrast, the single knockouts of NoxA, B, C and p22phox showed impaired ROS production under stimulation, indicating that NOXs are also important for ROS generation in Dictyostelium.

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