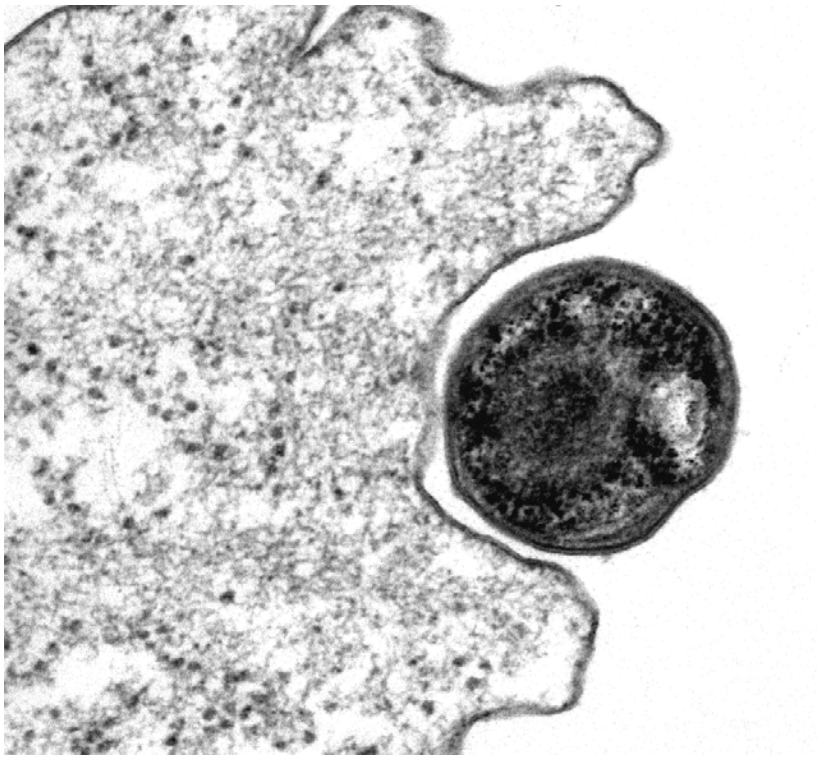
NEMO 2008

NON-MAMMALIAN EXPERIMENTAL MODELS FOR THE STUDY OF BACTERIAL INFECTIONS



Phagocytosis of a *Chlamydia*-related bacteria by the *Acantahmoeba* amoeba, as seen by electron microscopy (Picture by G. Greub, Lausanne, Switzerland)

Progress Report 2008

NEMO :
NON-MAMMALIAN EXPERIMENTAL MODELS FOR THE STUDY OF BACTERIAL INFECTIONS

Coordinator: Pr. P. Cosson

To test the ability of a bacteria to cause a disease it is usually necessary to infect a mammalian host and allow the disease to progress. These experiments inflict significant suffering to the animals. Our general aim is to stimulate the emergence of a community of scientists using alternative non-mammalian hosts for the study of bacterial infections. Our conviction is that many experiments currently carried out using mammalian hosts could be advantageously replaced by the use of alternative non-mammalian hosts. Our network of laboratories was created in Feb 2005, initially as an informal gathering of research groups involved in similar subjects. Our specific goals are:

1 - To organize an annual meeting on the theme of Non-mammalian hosts for the study of bacterial infections, in order to stimulate exchanges among research groups.

2 - To strengthen our research in this field through a series of collaborative works, for which we hope to find Swiss and European financial support.

3 - To publicize the use of alternative non-mammalian hosts in the scientific community.

The five research groups currently implicated in this network are:

Pierre Cosson, Geneva, CH (Pierre.Cosson@medecine.unige.ch)
Marie-Odile Fauvarque, Grenoble, FR (FAUVARQUEMARRASMO@dsvsud.cea.fr)
Gilbert Greub, Lausanne, CH (Gilbert.Greub@chuv.ch)
Hubert Hilbi, Zurich, CH (hubert.hilbi@micro.biol.ethz.ch)
Thierry Soldati, Geneva, CH (Thierry.Soldati@biochem.unige.ch)

In this Progress Report we describe briefly the results obtained in 2008.
A summary of the 2008 NEMO meeting is also attached.

Pseudomonas aeruginosa virulence genes identified in a *Dictyostelium* host model.

Pierre Cosson, Centre Médical Universitaire, University of Geneva;
Pierre.Cosson@medecine.unige.ch

SUMMARY

The human pathogen *Pseudomonas aeruginosa* has been shown previously to use similar virulence factors when infecting mammalian hosts or *Dictyostelium* amoebae. Here we randomly mutagenized a clinical isolate of *P. aeruginosa*, and identified mutants with attenuated virulence towards *Dictyostelium*. These mutant strains also exhibited a strong decrease in virulence when infecting *Drosophila* and mice, confirming that *P. aeruginosa* makes use of similar virulence traits to confront these very different hosts. Further characterization of these bacterial mutants showed that TrpD is important for the induction of the quorum-sensing circuit, while PchH and PchI are involved in the induction of the type III secretion system. These results demonstrate the usefulness and the relevance of the *Dictyostelium* host model to identify and analyze new virulence genes in *Pseudomonas aeruginosa*.

Pseudomonas aeruginosa is a gram-negative opportunistic pathogen and a major cause of nosocomial infections. This bacterium infects burn victims, intubated, immunocompromised and cystic fibrosis patients as well as users of soft contact lens. Its pathogenicity has been linked to at least two major virulence pathways, the quorum sensing (QS) system and the type III secretion system (TTSS). A comprehensive study of the pathways controlling bacterial pathogenicity is essential in understanding the mechanisms by which *P. aeruginosa* establishes harmful infections. However the assessment of bacterial pathogenicity in mammalian systems is limited by ethical and practical restrictions on animal experiments. To circumvent these limitations, *Pseudomonas* virulence can be assessed using *Dictyostelium* cells.

In order to identify new virulence genes we mutagenized a Pseudomonas patient strain, and tested the ability of *Dictyostelium* to grow on 2500 individual mutants. *Dictyostelium* was capable of growing on 17 of these mutants, which were further characterized in this study. The site of transposon insertion was determined for all 17 mutants. Sequence analysis identified 15 different transposon insertions in the *pchEFGHI* gene cluster: six in *pchE*, six in *pchF*, two in *pchH* and one in *pchI*. Another mutant was created by an insertion in the *pchDCBA* operon (*pchD* gene), localized in the same region.

To determine whether the isolated mutants presented a reduced virulence in other host systems, they were used to infect *Drosophila* flies, a well-characterized model for bacterial infections. In early infection experiments, flies were pricked with a needle previously dipped in a bacterial solution. More physiological methods of infection by *P. aeruginosa* have been developed more recently, where flies are fed with a sucrose solution containing bacteria. When *Drosophila* flies were fed with a solution containing the parental *P. aeruginosa,* or a

pchE mutant, 90% of them died within 8 days (Fig. 1). On the contrary, when *pchH*, *pchI* or *trpD* mutants were used, at least 90% of the *Drosophila* survived until the end of the experiment (Fig. 1). This result indicated that these three mutant strains isolated in a Dictyostelium host model were less virulent towards *Drosophila* than the parental strain.

These results demonstrate that an alternative non-mammalian host can be used to identify new bacterial virulence genes important in the infection of metazoan hosts.

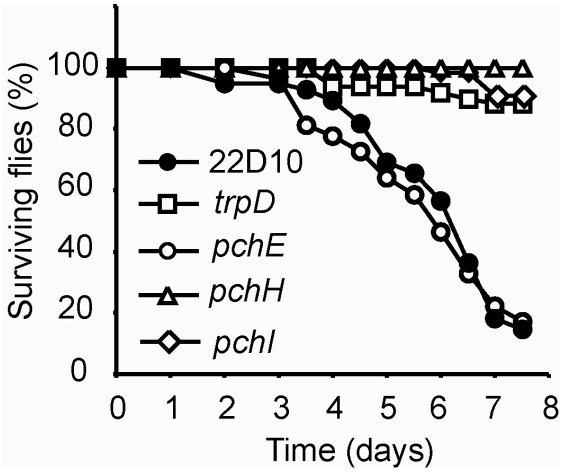


Figure 1. Virulence of *P. aeruginosa* mutants towards *Drosophila*.

Drosophila flies were fed with a sucrose suspension containing *P. aeruginosa* bacteria (parent or mutant, as indicated), and their survival was assessed twice a day for one week. At each time following infection the percentage of surviving flies is indicated. Mutants affected in *pchH*, *pchI* or *trpD* showed a reduced virulence towards *Drosophila*, and largely overlapping survival curves. The experiment was repeated three times with similar results, and one representative set of results is shown. When compared using the log-rank test, survival after infection with wild-type (22D10) was statistically different from survival after infection with *pchH*, *pchI* or *trpD* mutants ($p<0.001$), but not with *pchE* ($p=0.93$).

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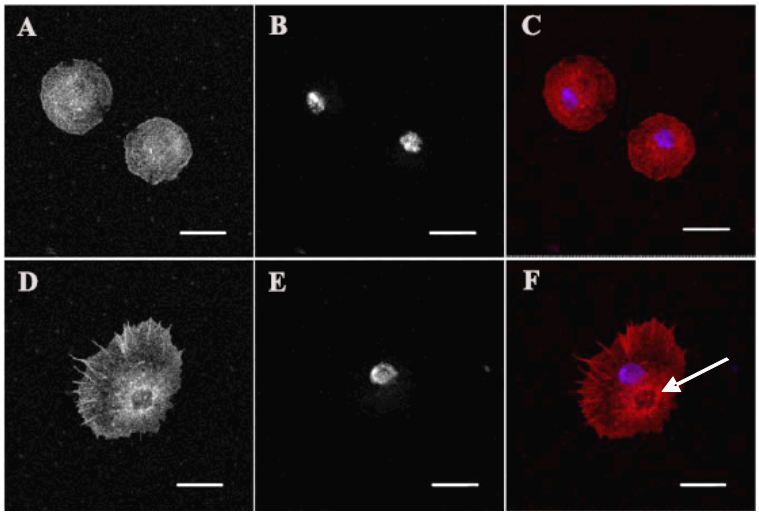
NONASPANINES FUNCTIONS IN *DROSOPHILA* CELLULAR IMMUNITY

Marie Odile Fauvarque, Laboratoire Transduction du Signal, iRTSV/LTS Inserm U873, CEA-Grenoble, 17 rue des Martyrs F-38054 France
marie-odile.fauvarque@cea.fr

Pathogen engulfment by host phagocytic cells and their subsequent killing in the phagocytic vacuole are major events for bacterial clearance and contribute to a robust innate immunity in most multicellular organisms. The unicellular phagocytic amoeba *Dictyostelium discoideum* has been used as a model organism to study and discover new genes implicated in phagocytosis. A genetic screen performed in Pierre Cosson's laboratory, identified *PHG1A* whose alteration causes a marked decrease in *Dictyostelium* adhesion to certain substrates and a strong impairment in bacterial phagocytosis and killing. *PHG1A* encodes a member of the TM9 protein family (also known as nonaspanins or TM9sf) characterised by the presence of nine transmembrane domains, and a high degree of evolutionary conservation. Thanks to its sophisticated immune system *Drosophila* represents a powerful host model for evaluating the contribution of phagocytic cells to host innate immunity. *Drosophila* has specialized circulating phagocytic cells derived from the hemocytic blood cell lineage. Plasmatocytes are the most abundant type of circulating hemocytes and represent the primary macrophages required for bacterial phagocytosis. Upon infection by parasites such as wasp eggs, plasmatocytes can recognize and attach to the invader and signal to the lymph gland to promote the differentiation of another kind of hemocyte called lamellocytes. These large cells attach to the plasmatocyte layer and form a hermetic capsule around the invader.
We performed the molecular characterization of the three *Drosophila* nonaspanins and the function in innate immunity of *DmTM9sf4*, the *Drosophila* ortholog of *Dictyostelium* Phg1A and human *hTM9sf4*. We have created *TM9sf4* null mutant flies and showed that their sensitivity to Gram-negative bacteria was correlated to impaired hemocyte-dependent phagocytosis. In addition, *TM9sf4* mutant larvae failed to properly encapsulate eggs from the avirulent wasp strain *Leptopilina boulardi* G486. These phenotypes are coupled to abnormal adhesion and defective cytoskeleton reorganisation in mutant plasmatocytes (Figure).

Confocal analysis of actin network in isolated larval macrophages



Control macrophages

***TM9sf4* mutant macrophages**

A,D: Phalloïdin fluorescent labelling revealed polymerised actin (Texas Red); B,E: Nuclei staining (Hoechst 33258) ; C,F : overlays. A-C: control cells present a regular size and a round shape. D-F Most of *TM9sf4* mutant cells present a larger area and differentiate long actin-stained filopodia

Our study shows that TM9sf4 function in cell adhesion and bacterial engulfment might result from defective cytoskeleton control and that TM9sf4 plays a crucial role in cellular immunity to ensure host defence against infections. TM9sf4 and TM9sf2, its closest paralog, were both required for bacterial internalization in S2 cells where they displayed partial redundancy.

Our study highlights the contribution of phagocytes to host defence in an organism possessing a complex innate immune response and suggests an evolutionary conserved function of TM9sf4 in eukaryotic phagocytes.

In parallel to this work, we used Drosophila to analyse the interaction of wild type and mutant pathogenic bacteria originally screened in *Dictyostelium* amoebae in Pierre Cosson's laboratory.

Publications 2008

Bergeret E, Perrin J, Williams M, Grunwald D, Engel E, Thevenon D, Taillebourg E, Bruckert F, Cosson P and Fauvarque MO (2008). TM9SF4 is required for Drosophila cellular immunity via cell adhesion and phagocytosis.. **J. Cell Science**, 15 : 3325-3234

Alibaud L, Köhler T, Coudray A, Prigent-Combaret C, Bergeret E, Perrin J, Benghezal M, Reimman C, Gauthier Y, vanDelden C, Attree I, Fauvarque MO and Cosson P. (2008) Pseudomonas aeruginosa virulence genes identified in a Dyctiostelium host model. **Cell microbiology**, 10:729-740

Amoebae as a tool to isolate new bacterial species, to test bacterial virulence and to study the biology of intracellular amoebae-resisting bacteria

Gilbert Greub, Institute of Microbiology, University of Lausanne, Switzerland (gilbert.greub@chuv.ch)

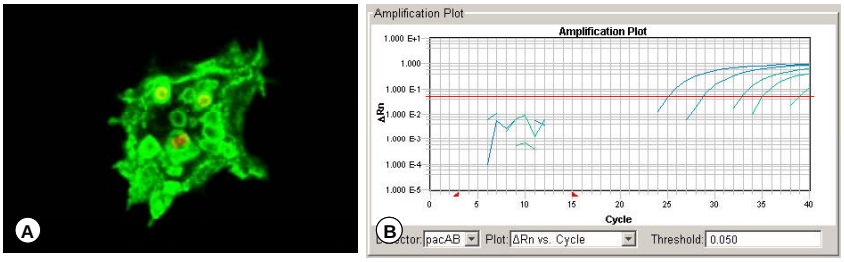
Free-living amoebae feed on bacteria, fungi and algae. However, some microorganisms evolved to become resistant to these protists. These amoebae-resistant microorganisms include established pathogens, such as *Legionella* spp. and *Mycobacterium* spp., as well as *Chlamydia*-related emerging pathogens. Our group use amoebae as a tool for the culture of intracellular bacteria: more precisely, we are using amoebae as cells in a cell culture system to study the biodiversity of intracellular hosts of free-living amoebae in water and since human are commonly exposed to water, the potential role played by these intracellular bacteria as agents of pneumonia (see a schema of our research program in (10)). Our research mainly focus on *Chlamydia*-like organisms, such as *Protochlamydia naegleriophila* (6), *Waddlia chondrophila* (2, 3) and *Parachlamydia acanthamoebae* (1, 4, 5, 7).

Recently, using amoebae as cell in a cell culture system, we successfully identified in complex microbiota several new members of the *Chlamydiales* order (8), as well as *Legionella* and Mycobacteria (11).

Animal models are generally used to test bacterial virulence. *Dictyostelium* also appear to be an excellent model to test the virulence of intracellular bacteria. We therefore decided to start a study that aim at investigating the interaction between the intracellular bacteria *Parachlamydia/Protochlamydia/Waddlia* and the *Dictyostelium* amoeba. In particular, we intend to determine the molecular mechanisms that control their interactions with the phagocytic cell. For this, we will determine which genes are important on the host side (*Dictyostelium*).

As a preliminary to host genetic analyses, we already defined the intracellular transport of *Parachlamydia* in *Dictyostelium*. Preliminary results indicate that internalized *Parachlamydia* are located in the endocytic pathway in *Dictyostelium* (Fig. 1A). We also set up conditions to quantify the number of *Parachlamydia* and *Protochlamydia* by quantitative PCRs (7), thus assessing its intracellular replication in *Dictyostelium* and other host cells (Fig. 1B). We are now ready to start analyzing the effect of various mutations in the host genome on the intracellular replication and trafficking of *Parachlamydia*.

Figure 1.
A. *Parachlamydia* (red) traffic within an endocytic vacuole (green) in *Dictyostelium*;
B. Growth of *Parachlamydia* from day 0 to day 4 quantified using a taqMan quantitative PCR



We also studied the intracellular traffic of another *Chlamydia*-related bacteria, *Protochlamydia neagleriophila* strain KNIC, which role in lung infections has just been identified (6). This bacterial species was easily replicating within *Dicytostelium,* as confirmed by immunofluorescence and quantitative PCR (Figure 2).

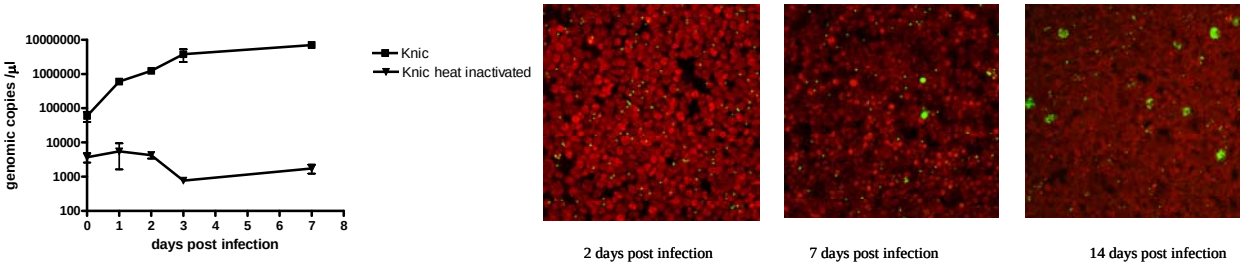


Figure 2. A specific quantitative PCR targeting the 16S rRNA gene of *P. neagleriophila* demonstrated the 2-log increase in the number of DNA copies in about 3 days when co-cultured with *Dictyostelium* (left panel); large inclusions were however mainly observed after 7 to 14 days of incubation (right panel).

Like *Parachlamydia acanthamoebae*, *Protochlamydia neagleriophila* strain KNIC co-localized mainly with markers of the endocytic pathway (p80) and to a lesser extend with markers of the contractile vacuole (Rh50, see Figure 3).

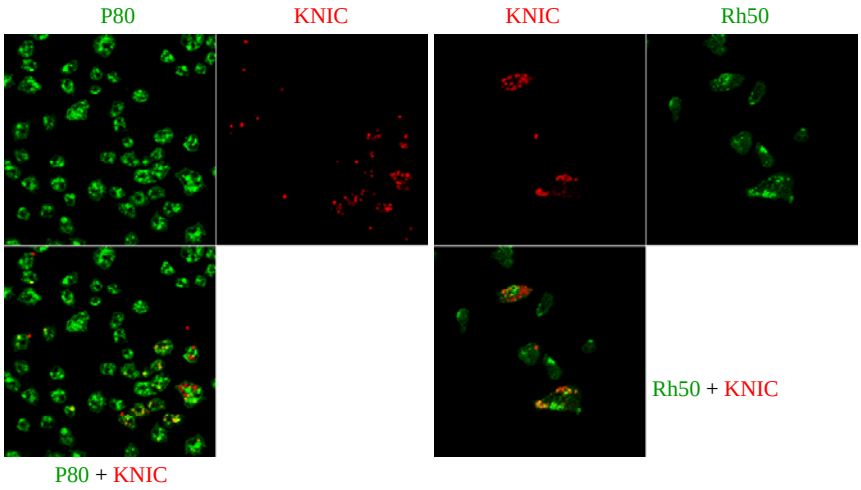


Figure 3. Co-localization of *P. neagleriophila* with markers of the endocytc pathway (p80, left panel) and with markers of the contractile vacuole (Rh50, right panel).

More recently, we demonstrated the ability of *Waddlia* to rapidly grow within human macrophages (9). This resistance to human macrophages is likely due to the escape of the bacteria from the endocytic pathway (Croxatto and Greub, submitted). We thus now intend to compare the intracellular traffic of *Waddlia* in both types of phagocytic cells: macrophage and the *Dictyostelium* amoeba.

In conclusion, our ongoing projects provide evidences on the usefulness of amoebae as tool to investigate the biology of intracellular bacteria resisting-amoebae. Amoebae appear to represent convincing alternative non-mammalian model to study bacterial virulence as well as the interactions of these bacteria with eucaryotic cells. The NEMO network provide a specific access to a variety of *Dictyostelium* mutants that may help in better understanding the host factors implied in determining the results of the encounter between the internalized bacteria and the phagocyte. Moreover, the NEMO community are helpful by providing a forum of discussion.

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AMOEBAE: A CELLULAR PATHOGENESIS MODEL FOR THE LEGIONNAIRES' DISEASE AGENT *LEGIONELLA PNEUMOPHILA*

Hubert Hilbi, Institute of Microbiology, ETH Zürich; hilbi@micro.biol.ethz.ch

SUMMARY

The opportunistic human pathogen *Legionella pneumophila* replicates within environmental amoebae as well as within macrophages in specific "*Legionella*-containing vacuoles" (LCVs). Formation of LCVs requires the bacterial Icm/Dot type IV secretion system and more than 100 effector proteins, which exploit host cell signal transduction and trafficking pathways. To understand the formation of LCVs on a molecular and cellular level, we analyze host cell factors and vesicle trafficking pathways implicated in LCV formation, Icm/Dot substrates subverting host pathways and virulence gene regulation of *L. pneumophila*. To this end, we purified intact LCVs from *Dictyostelium* amoebae by immuno-magnetic separation and determined the proteome of the vacuoles (Urwyler *et al*., 2009, Fig. 1). Thus, more than 560 proteins were identified including small GTPases implicated in the secretory and endosomal vesicle trafficking pathways. The recruitment of these GTPases to LCVs was confirmed using GFP fusion proteins.

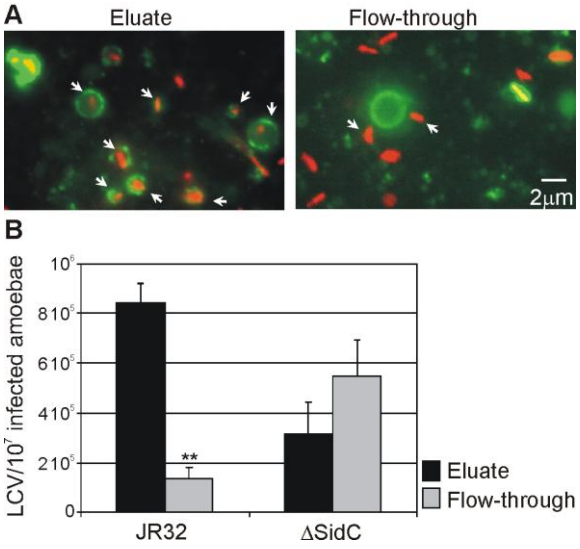

Fig. 1. Enrichment of *Legionella*-containing vacuoles by immuno-magnetic separation. *Dictyostelium* producing calnexin-GFP was infected with *L. pneumophila* expressing the red fluorescent protein DsRed and lysed. Intact LCVs were enriched by immuno-magnetic separation using an antibody against the LCV-bound *L. pneumophila* effector protein SidC and a secondary antibody coupled to magnetic beads. (**A**) Fluorescence images of intact LCVs (eluate, arrows) and free bacteria (flow-through, arrows). (**B**) Quantification of the enrichment. LCVs containing wild-type *L. pneumophila* JR32 but not a Δ*sidC-sdcA* mutant strain were enriched in the eluate approximately 7 times. ** $P < 0.005$.

To analyze LCV formation, we currently focus on the host cell phosphoinositide (PI) metabolism. PIs are pivotal regulators of eukaryotic signal transduction and vesicle trafficking pathways. Using *Dictyostelium* amoebae lacking individual PI 5-phosphatases we found that *L. pneumophila* replicates 2-3 orders of magnitude more efficiently in absence of the PI 5-phosphatase Dd5P4, and hence, Dd5P4 is a resistance factor of *Dictyostelium* (Weber *et al*., 2009). Dd5P4 is a homologue of the mammalian enzyme OCRL1 (Oculocerebrorenal syndrome of Lowe), which is implicated in retrograde vesicle trafficking from endosomes to the Golgi apparatus. Interestingly, Dd5P4 as well as OCRL1 localize to LCVs in amoebae or macrophages, respectively, *via* an N-terminal domain previously not implicated in membrane localization.

We previously identified the *L. pneumophila* Icm/Dot substrate SidC as a protein binding to phosphatidylinositol 4-phosphate (PtdIns(4)*P*) on the LCV membrane. The PtdIns(4)*P*-binding domain of SidC was now mapped to a 20 kDa fragment termed "P4C" (PtdIns(4)*P*-binding of SidC) domain and does not show similarity to eukaryotic PI-binding domains (Ragaz *et al*., 2008). Moreover, we identified the Icm/Dot-translocated Rab1 guanine nucleotide exchange

factor (GEF) SidM/DrrA as another major *L. pneumophila* PtdIns(4)*P*-binding effector protein (Brombacher *et al*., 2009). SidM harbours a novel 10 kDa PtdIns(4)*P*-binding domain termed "P4M" (PtdIns(4)*P*-binding of SidM/DrrA) and competes with SidC for binding to PtdIns(4)*P* on LCVs. In contrast, the Icm/Dot-translocated Arf1 GEF RalF does not bind to any PIs, indicating that *L. pneumophila* produces two classes of GEFs that either bind to PIs or not.

L. pneumophila adopts a biphasic life style consisting of a replicative and a stationary (transmissive/virulent) phase. We previously identified a gene cluster encoding a putative quorum sensing autoinducer synthase (*lqsA*), a sensor kinase (*lqsS*) and a novel response regulator (*lqsR*), which is a component of the stationary phase virulence gene regulatory network (Fig. 2). We now identified the signaling molecule produced by LqsA as 3-hydroxy-pentadecane-4-one (Spirig *et al*., 2008). Moreover, compared to an *lqsR* deletion mutant, a strain lacking all 4 genes of the *lqs* cluster showed more pleiotropic and more severe phenotypes, suggesting synergistic and complex interactions among the *lqs* and other regulatory genes (Tiaden *et al*., 2008).

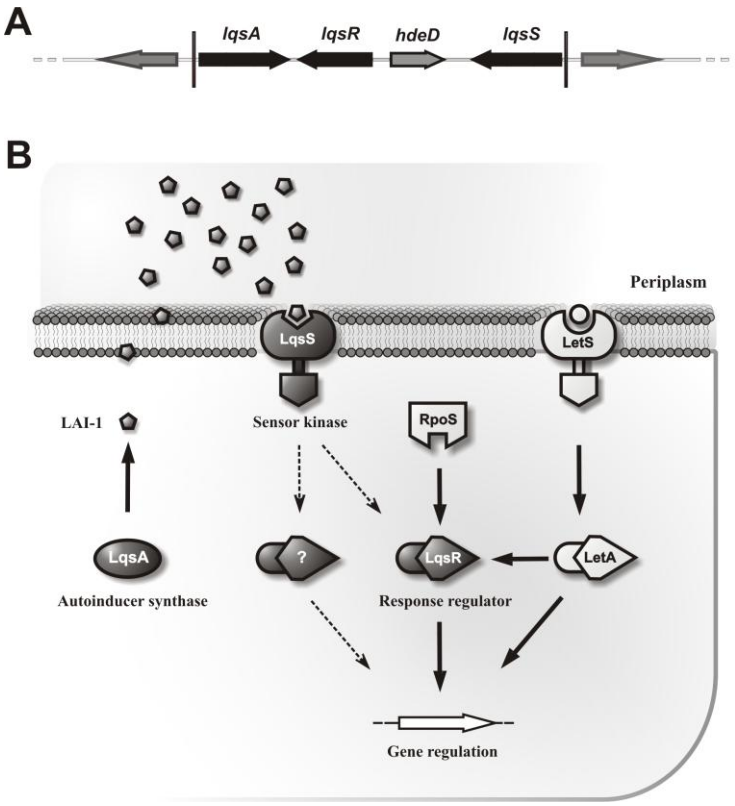

Fig. 2. The *lqs* gene cluster and model of the *L. pneumophila* quorum sensing circuit. (**A**) Chromosomal map of the *L. pneumophila lqs* gene cluster. The autoinducer LqsA and sensor kinase LqsS are 45% and 29% identical to *V. cholerae* CqsA and CqsS, respectively. (**B**) Model of the *L. pneumophila* autoinducer circuit, including LqsA, the low molecular weight diffusible signaling molecule LAI-1 (*Legionella* autoinducer-1), the cognate sensor kinase LqsS and the response regulator LqsR. The expression of LqsR is controlled by the alternative sigma factor RpoS and the two-component system LetA/LetS. Dashed lines indicate putative pathways and links.

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THE AMOEBA *DICTYOSTELIUM DISCOIDEUM* AS A MODEL HOST TO STUDY MYCOBACTERIUM MARINUM INFECTION

Thierry Soldati, Department of Biochemistry, University of Geneva
thierry.soldati@unige.ch

1. Establishment of *Dictyostelium* as a host model to study mycobacteria infection

Mammalian host model systems, whole organisms as well as cell lines, are essential to understand the pathogenicity of mycobacteria. However, over the past decades the complexity of these systems has hampered advancement to unravel the dynamic host-pathogen interactions. Pathogenic mycobacteria such as *M. tuberculosis*, *M. marinum* and *M. leprae* utilise common strategies to invade phagocytes of the innate immune system, reprogram their otherwise bactericidal phagosome maturation pathway to establish a friendly replication niche. *M. marinum* is the closest relative to the tuberculosis group of mycobacteria and provides a powerful model to study the pathogenesis of tuberculosis in genetically tractable model organisms, such as *Drosophila* and zebrafish. Using the soil amoeba *Dictyostelium discoideum* as a host, we have identified and characterized mycobacterial and host factors that modulate resistance to infection and cell-to-cell spreading (Hagedorn & Soldati, Cell Microbiol, 2007, 9:2716-33).

2. Identification of novel host factors that modulate cell-intrinsic immunity

To characterize the course of infection at the single cell, population and functional levels, we applied a combination of approaches, including FACS, IFA and EM analyses.

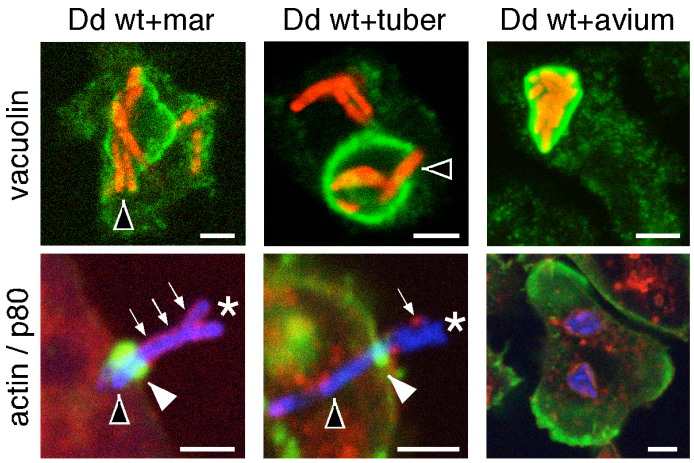
By testing a collection of *D. discoideum* mutants we identified factors important for infection with *M. marinum*. NRAMP (Natural Resistance-Associated Macrophage Protein, an iron pump) is an evolutionarily conserved endosomal membrane protein. The Nramp1 ko is more susceptible to infection and shows an impaired recruitment of the V-ATPase to the bacteria-containing proliferation vacuole. Even an avirulent *M. marinum* mutant (L1D) is able to manipulate the Nramp1 ko to some extent, whereas the gram-negative *K. aerogenes* is quickly killed. GFP-Nramp1 localizes to the early bacteria-containing compartment suggesting a direct role. The lack of LmpA or LmpC -members of the CD36 scavenger membrane receptor family residing in phago-lysosomes - renders *D. discoideum* mutants more susceptible to infection with *M. marinum*. Knock out of LmpB, the only family member residing also at the plasma membrane does not increase the susceptibility to infection. However, both the LmpA and LmpB mutants show a strong specific defect in uptake of *M. marinum* (Sattler et al, in preparation)

We also showed that the absence of RacH - a small GTPase RacH that regulates vacuolin distribution in the endocytic pathway - renders the host more susceptible to *M. marinum* proliferation but inhibits the non-lytic intercellular spreading of cytosolic bacteria via ejectosomes.

3. The ejectosome: a novel, nonlytic mechanism of pathogen release from its cell host

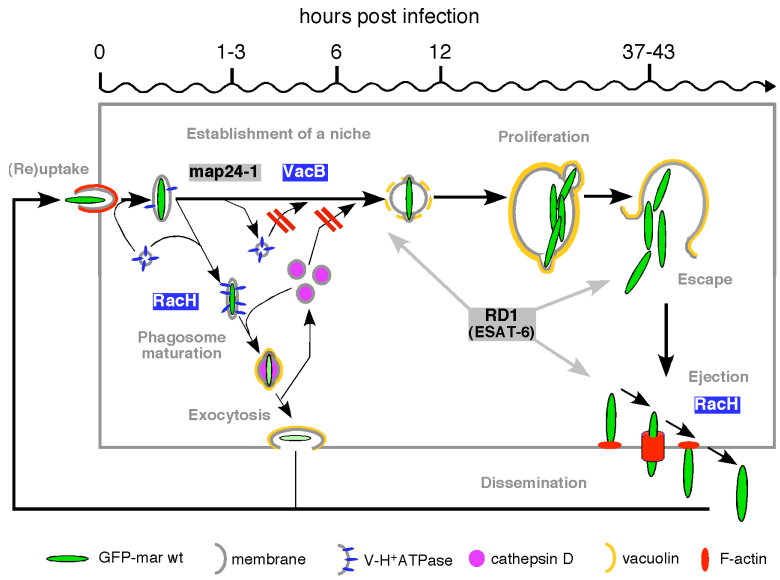
Pathogen release from its host cell is an important virulence determinant and an aspect of host-pathogen interactions that has long suffered from neglect. We discovered that both *M. marinum* and *M. tuberculosis*, but not *M. avium*, can escape from their vacuole into the cytosol, and are ejected from the cell through an F-actin structure, the ejectosome (**see Fig 1,** Hagedorn et al, 2009, in press). This appears to be a conserved strategy because, upon infection of human peripheral blood monocytes and murine microglial cells, flotillin-1 accumulation at the *M. marinum* replication niche and vacuole rupture were also observed. Upon ejection of *M. marinum*, despite local loss of membrane integrity, neither host cell leakage nor lysis was observed. Ejection is crucial for the maintenance of an infection and is a concerted process that requires both host and pathogen factors.

Figure 1



Disruption of the *Dictyostelium* gene for the RacH GTPase led to intracellular accumulation of bacteria. Also, no ejectosome was formed in cells infected with *M. marinum* lacking the major conserved mycobacterial virulence locus RD1. The defect could be trans-complemented by expressing the small secreted mycobacteria virulence effector ESAT-6 directly in the cytosol of the *Dictyostelium* host cell (**see Fig 2**). We propose that the specific ejection strategy evolved as a necessity for the release of a cytosolic pathogen in a mutually beneficial manner, and discuss its evolutionary origin and relevance for dissemination of a mycobacterial infection (Hagedorn et al, 2009, in press).

Figure 2



4. Publications for the year 2008

1. Hagedorn, M., Kyle H. Rohde, David G. Russell, and **Soldati**, T. Infection by tubercular mycobacteria is spread by nonlytic ejection from their amoeba hosts. **Science** (2009) in press
2. Dieckmann, R., Gopaldass, N., Escalera, C., and **Soldati**, T. Monitoring time-dependent maturation changes in purified phagosomes from *Dictyostelium discoideum*. **Methods Mol. Biol.** (2008) 445, 327-337.
3. Dieckmann, R., and **Soldati**, T. Phagosome proteomes unite! A virtual model of maturation as a tool to study pathogen-induced changes. In **Intracellular Niches of Microbes:** A Pathogens Guide Through the Host Cell, Ed Schaible, U.E., and Haas, A. John Wiley & Sons, Ltd, (2008) in press.
4. Hagedorn, M., and **Soldati**, T. *Mycobacterium marinum*. In **Intracellular Niches of Microbes:** A Pathogens Guide Through the Host Cell, Ed Schaible, U.E., and Haas, A. John Wiley & Sons, Ltd, (2008) in press.
5. Cosson, P, and **Soldati**, T., Eat, kill or die: when amoeba meets bacteria. Invited review for **Current Opinion Microbiol.** (2008) 11, 271-276

NEMO meeting 2008 report

Non-mammalian Experimental Models for the study of bacterial infection
Supported by the Swiss 3R Foundation
Department of Biochemistry
University of Geneva

The annual 2008 NEMO meeting took place in Geneva on April 24th and 25th. A series of international group leaders described how non-mammlian models can be used in a variety of situations as surrogate hosts to study bacterial infections. The practical advantages of alternative models were evident in many presentations, and led to the discovery of many new aspects of bacterial pathogenesis. Potential new antibacterial agents were also studied in some alternative models.

Dr. Astrid van der Saar (Amsterdam, The Netherlands) described the strong similarities between Mycobacterium tuberculosis and Mycobacterium marinum. M. tuberculosis is a major helath problem world-wide, but its study is difficult for security reasons. Mammalian models do not recapitulate perfectly the disease as observed in infected patients. M. marinum naturally infects fish and can thus be studied advantageously using Zebrafish embryos as surrogate models for mammals. Indeed, infection leads to the formation of typical granulomas. Using this model, four new bacterial virulence genes were identified. Host genes involved in resistance to infections can also be studied in this non-mammalian model.

Dr. Monica Hagedorn (Geneva, Switzerland) showed how virulence of M. marinum can also be assessed using Dictyostelium unicellular amoebae as surrogate hosts. Virulent mycobacteria replicate inside host cells, disrupt the phagosomal membrane and gain access to the cytosol. They then escape the cells by a newly-characterized mechanism allowing bacteria to cross the cell membrane without killing the cell.

Response to infections can also be studied using Drosophila as a host. Dr. Dominique Ferrandon (Strasbourg, France) studied how the humoral antimicrobial response is induced in flies by a septic injury. Fungi, gram-negative bacteria and gram-positive bacteria activate differentially two distinct pathways, resulting in adequate humoral responses. Serratia marescens, an emerging human pathogen was studied in this system. When fed to flies, it escapes the gut, except when the production of proteases is affected. The mechanisms by which S. marescens avoids induction of a systemic response are studied in this system.

Dr. Jackie Perrin (Fauvarque's group, Grenoble, France) studied the function of nonaspanins in various aspects of the immune response of Drosophila. Analysis of nonaspanin mutants revealed their role in phagocytosis and cellular immunity. Although these genes exist in mammals, their role can be advantageously studied in genetically tractable organisms like Drosophila.

Dr. Romain Froquet (Cosson's group, Geneva, Switzerland) showed how Dictyostelium amoebae can be used to measure virulence of various bacterial pathogens as an alternative to infecting mammals. Experimental conditions can be

adjusted to measure virulence of a wide range of bacteria, revealing the extreme versatility of this alternative model.

Dr. Siouxsie Wiles (London, Great Britain) showed how bioluminescence can be used to study bacterial pathogens during an infection. This technique provides real-time information about infections. This technique also represents a significant refinement of current procedures used in infected animals since it is non-invasive and could thus reduce significantly the number of animals used in each experiment where bacterial infections are studied.

Dr. Antony Croxatto (Greub's group, Lausanne, Switzerland) studied Waddlia chondrophila, an emerging pathogen presumably responsible for miscarriages in cultured human cells. Once endocytosed, Waddlia blocks maturation of the phagosome and escapes to the ER where it replicates. Newly-developed inhibitors of the type-III secretion system inhibit intracellular growth of Waddlia and could thus be used to study the biology of this strict intracellular bacteria.

Dr. Elzbieta Wyroba (Warsaw, Poland) uses Paramecium to study intracellular membrane trafficking. Her studies reveal a potential role for rab7 in transport from early endosomes to late endosomes, and possibly to later endocytic compartments. The potential role of dynein in this process was presented.

Dr. Hubert Hilbi (Zürich, Switzerland) made use of amoeba as a host to study Legionella infections. Using icm/dot mutants of Legionella, he revealed the critical role of cellular PI3 kinase and of PI5P phosphatase in controlling bacterial replication. The role of bacterial SidC was analyzed in detail. This protein binds to PI4P on the Legionella-containing vacuole and plays a role in recruitment of endoplasmic reticulum.

Dr. Gunmar Sandström (Stockholm, Sweden) discussed how the natural environment of bacteria differs from conditions used in laboratory experiments. One survival strategy for pathogens in the environment is to infect non-mammalian hosts such as Acanthamoebae and replicate intracellularly. Understanding the ecology of pathogens like Francisella, V. cholera or Shigella in the environment will allow to better understand the appearance of epidemic situations.