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Inhibition of apoptosis and NF-κB activation by vaccinia virus protein N1 occur via distinct binding surfaces and make different contributions to virulence

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Vaccinia virus (VACV) protein N1 is an intracellular virulence factor that belongs to a family of VACV B-cell lymphoma (Bcl)-2-like proteins. Members of this family inhibit apoptosis or activation of pro-inflammatory transcription factors, such as interferon (IFN) regulatory factor-3 (IRF-3) and nuclear factor-κB (NF-κB). Remarkably, N1 inhibits both apoptosis and NF-κB activation.

To understand how N1 exerts these different functions, we have mutated residues in the Bcl-2-like surface groove and at the interface used to form N1 homodimers. Introduction of bulky residues in the Bcl-2-like groove abolished only the N1 anti-apoptotic activity, and its interaction with cellular pro-apoptotic Bcl-2 proteins such as Bad or Bid. Protein crystallography showed these mutants differed from wild-type N1 only at the site of mutation. Conversely, mutagenesis of the dimer interface converted N1 to a monomer and affected only inhibition of NF-κB activation. Collectively, these data show that N1 inhibits pro-inflammatory and pro-apoptotic signalling using independent surfaces of the protein.

To determine the relative contribution of each activity to virus virulence, mutant N1 alleles were introduced into a VACV strain lacking N1 and the virulence of these viruses was analysed after intradermal and intranasal inoculation in mice. In both models, VACV containing a mutant N1 unable to inhibit apoptosis had similar virulence to wild-type virus, whereas VACV containing a mutant N1 impaired for NF-κB inhibition induced an attenuated infection similar to that of the N1-deleted virus. This indicates that anti-apoptotic activity of N1 does not drive virulence in these in vivo models, and highlights the importance of pro-inflammatory signalling in the immune response against viral infections.
Ergothionein and the glutathione analogs ophthalmate and norophthalmate: from mammals to cyanobacteria

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Cyanobacteria, the only known prokaryotes that perform oxygenic photosynthesis, are regarded as being (i) amongst the oldest life forms on earth; (ii) the producers of the Earth’s oxygenic atmosphere; and (iii) the progenitor of the plant chloroplast. Over time, cyanobacteria have evolved as the most diverse group of bacteria that colonize most biotopes (fresh, brackish and marine waters, and soils). The hardness of cyanobacteria is due to their efficient photosynthesis that uses nature’s most abundant resources, solar energy, water, CO2 and mineral nutrients, to produce a large part of the atmospheric oxygen and organic assimilates for the food chain. Cyanobacteria fix annually about 25 Giga tons of carbon from CO2 into energy dense biomass. For this, cyanobacteria use about 0.3% of the solar energy, 178,000 TW, reaching the Earth surface, a value exceeding by more than 25 times the energy demand of the human society (about 15 TW). Furthermore, the availability of molecular tools for gene manipulation make cyanobacteria promising “low-cost” microbial cell factories for the carbon-neutral production of biofuels, while saving arable soils for crops.

In producing the oxygenic atmosphere, cyanobacteria were the first organisms to face oxidative stress and iron limitation (due to iron oxydation), which is especially detrimental to the iron-requiring photosynthesis pathway. Consequently, cyanobacteria have developed powerful strategies to cope with the oxidant byproducts generated by their active photosynthesis and respiration. Many of these strategies were conserved by evolution such as the production of glutathione (γ-L-glutamyl-L-cysteinyl-glycine), which was regarded merely as a redox buffer until we and other groups showed that it also operates in the assembly of the iron-sulfur cluster of anti-oxidant glutaredoxin enzymes.

Using a systems biology approach* (functional genomics, transcriptomics, metabolomics etc..) we thoroughly analyzed of the role of glutathione synthesis (GshA and GshB enzymes) and degradation (Ggt) in the physiology and tolerance to metal and oxidative stresses in two model cyanobacteria. Among other results we report, for the first time, that GshA and GshB also synthesize the two GSH analogs, ophthalmate (γ-L-glutamyl-L-α-amino-n-butyryl-glycine) and norophthalmate (γ-L-glutamyl-L-alanyl-glycine), which we had been described only in mammals so far. We will discuss the role ophthalmate and norophthalmate in stress signalling (ie; sulfur status or starvation) and in both amino-acid and sugars metabolisms.

We also report, for the first time that some cyanobacteria are able to synthesize another sulfur-containing antioxidant: ergothionein (EGT), which was also described only in mammals, which acquire EGT solely through their diet. We will discuss the reciprocal role of GSH in the synthesis EGT and its interplay in the tolerance to oxidative and metal stresses.

Mechanisms underlying the switch from rods to L-forms in Bacillus subtilis

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The cell wall is a crucial protective layer that surrounds virtually all bacteria. However, some bacteria have developed mechanisms to switch between walled and wall-deficient states. These cell wall-deficient bacteria, known as L-forms, have been isolated from many different species. They represent an interesting model of study considering their potential involvement in antibiotic resistance and persistent infections. But despite decades of study the mechanisms underlying cell division in the absence of the cell wall are poorly understood. We have isolated a strain of Bacillus subtilis that can quickly and quantitatively convert from the walled to the L-form state. Mutations in two different genes contribute to the high frequency of L-form transition: walR, a transcriptional regulator involved in cell wall homeostasis; and sepF, required for accurate and efficient cell division. Time-lapse imaging shows that the mutations act by facilitating the release of the L-form from its walled parent cell but that they act in different ways. In a previous work, we showed that L-form division does not require FtsZ or any residual peptidoglycan synthesis. In fact, many essential proteins for the cell walled form have turned out to be dispensable for L-form survival and proliferation. Therefore, we conducted a genetic screen using random transposon mutagenesis to identify genes exclusively essential for L-form division. Our results have shown the crucial role of membrane lipid composition and its biophysical properties during L-form division.


3. Domínguez-Cuevas, P., Mercier, R., Leaver, M., Kawai, Y., Errington, J. Molecular Microbiology. 2012 Jan; 83(1):52-66. The rod to L-form transition of Bacillus subtilis is limited by a requirement for the protoplast to escape from the cell wall sacculus.


The YvcK protein is required for morphogenesis via localization of PBP1 under gluconeogenic growth conditions and is regulated by Ser/Thr phosphorylation in Bacillus subtilis.

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The YvcK protein was previously shown to be dispensable when B. subtilis cells are grown on glycolytic carbon sources but essential for growth and normal shape on gluconeogenic carbon sources. YvcK is localized as a helical-like pattern in the cell. This localization seems independent of the actin-like protein, MreB. A YvcK overproduction restores a normal morphology in an mreB mutant strain when bacteria are grown on PAB medium. Reciprocally, an additional copy of mreB restores a normal growth and morphology in a yvcK mutant strain when bacteria are grown on a gluconeogenic carbon source like gluconate. Furthermore, as already observed for the mreB mutant, the deletion of the gene encoding the penicillin-binding protein PBP1 restores growth and normal shape of a yvcK mutant on gluconeogenic carbon sources. The PBP1 is delocalized in an mreB mutant grown in the absence of magnesium and in a yvcK mutant grown on gluconate medium. Interestingly, its proper localization can be rescued by YvcK overproduction.

Recently, we observed a new connection between carbon metabolism and cell shape regulation in Bacillus subtilis. The enzymatic properties YvcK are unknown, but YvcK possesses a Rossmann fold, and is an NAD(P)(H) binding protein. Furthermore, YvcK, like its Mycobacterium tuberculosis counterpart, is phosphorylated on a threonine residue. The overproduction of protein modified at the phosphorylation site (phosphoablative YvcK) cannot rescue an mreB mutant and PBP1 is still mislocalized. This mislocalization is also observed in an mreBprkC mutant that overproduces YvcK. How YvcK influences the localization or activity of PBP1 is unknown but, YvcK joins the increasing number of proteins with an enzymatic signature that affect cellular processes.
A metabolomics approach to study bacterial virulence in C elegans reveals distinct metabolic profiles

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There remains a need for robust and simple animal models of infection to allow for better understanding of host-microbe interactions, bacterial pathogenesis as well as development of antimicrobial therapies. An attractive model is the invertebrate nematode *Caenorhabditis elegans*, now well recognized as relevant for the study of bacterial pathogenesis as well as a model for other conditions. The worm *C.elegans* is susceptible to a number of bacterial pathogens, which are able to kill or induce a range of symptoms of disease. Human pathogens already known to affect *C. elegans* include Gram-negative bacteria such as, Pseudomonas and Salmonella as well as a number of Gram-positive bacteria such as Enterococcus and Staphylococcus. Many of these are able to colonize the worm intestine, and the pathogenic effect can be measured by marking the decrease in lifespan of the nematode. Several studies have already been undertaken in which mutant libraries of a bacterial pathogen have been tested in the worm to identify candidate virulence genes. *C. elegans* also offers researchers a simpler model for analysis of the host side of pathogen-host interactions. Genetic analysis of the host is relatively straightforward, for example by screening for worm mutants either more resistant or hypersensitive to a given pathogen. In this study we have used the *C. elegans model* coupled with direct infusion Ion cyclotron resonance Fourier transform mass spectrometry (DI-ICR-FT/MS) to investigate the unique metabolic phenotypes in worms facing specific stresses, like infection or starvation. Metabolomics is an analytical option for the global analysis of metabolites within a biological sample, providing a direct read out of an organism’s metabolic state. These read outs are condition-dependent and thus changes in given metabolite concentrations mirror changes in physiological conditions in response to stimuli. Our aim was to undertake whole worm metabolomics and recover markers of the induced metabolic changes in *C elegans* brought on by interaction with pathogens. In this investigation, we utilize this model system to reveal complex metabolic phenotypes allowing clustering based upon challenge. We identify general end product indicators of bacterial infection within this system, as well as specific markers for each of the experimental challenge conditions.
The def operon overproduced/expressed in Bacillus subtilis suppresses the mta deletion

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In Bacillus subtilis, the Mta Multidrug transporter activator regulates the expression of bmr and blt, which encode two multidrug efflux transporters. Mta was found here to play an unexpected role in central physiological processes, since the absence of this activator results in low levels of CpgA, a P-loop GTPase, and this effect is suppressed by a secondary mutation, which increases the levels of CpgA and improves the fitness of the resulting cells. The higher levels of CpgA in these cells most probably proceeds from an increase of the expression of the def operon which comprises seven genes, def, fmt, yloM and yloN, encoding a peptide deformylase, the N-formyltransferase, an E. coli RsmB homologous and a protein of unknown function belonging to the RlmN family respectively, all of which certainly or probably involved in the translation process, and prpCprkCcpgA, which encodes a Ser/Thr signaling system involved in peptidoglycan expansion or deposition. This up expression of the def operon (Pdef up) yields hyperchained non motile cells. Time lapse experiments showed that the mta mutation has no effect on the ON/OFF switch governing cell chaining and motility and is reversible like in wild type cells. However, mta Pdef up strains are locked in a state in which the cell separation and motility genes are in the OFF state. The SigD transcriptional factor is present and active in the mta strain, while its activity is drastically reduced in the mta Pdef up mutant, although the protein level is not affected by the Pdef up mutation. The fact that IPTG-induced Pspacdef in a Mta+ background results in a permanent OFF state shows that an hyperchaining phenotype could be obtained with an up expression of the def operon only.
Genetic control of dynamic polarity in *Myxococcus xanthus*

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* *Myxococcus xanthus* is a social bacterium with a complex developmental lifestyle. Motile Myxococcus cells cooperate by motility and their ability to change their direction of movement in a process where the poles exchange roles, allowing the cells to resume movement in the opposite direction, in a highly regulated manner. A small G-protein MglA was described to activate the motility machinery in *M. xanthus* and to change its polar localization to drive cellular reversals. This Ras-like small G protein oscillates between active (GTP-bound) and inactive (GDP-bound) states to control the switch. The GTPase-Activating Protein MglB increases the hydrolysis of GTP by MglA to reach its inactive state. Pole to pole switching of these two motility proteins is controlled by the Frz signal transduction pathway, a bacterial chemosensory-like system where a soluble receptor activates a histidine kinase, which phosphorylates a response regulator to trigger reversals. Previously, it was shown that the response regulator FrzZ is phosphorylated by the Frz kinase, however how FrzZ is linked to the MglAB system has remained mysterious.

In this study, we are trying to elucidate how the Frz cascade is connected to the MglA and MglB switch complex. We first found that a new response regulator protein RomR controls the polar localization of MglA in a Frz-dependent system. In this study, we show ongoing genetic, cell biology and biochemical approaches to elucidate how FrzZ, RomR, MglA and MglB cooperate to provoke the polarity switch. This way, we hope to model how external signals are integrated to control motility and ultimately generate cooperative behaviors.
Development of Signature Tagged Mutagenesis of *Lactobacillus casei* to identify genes implied in gut persistence.


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*Lactobacillus casei* is a well-documented probiotic species concerning its benefit for the host, but mechanisms involved in the symbiotic interactions with the host are poorly understood. This is notably due to the lack of reliable genetic tools to perform reverse genetics in lactobacilli. The aim of this study was to develop a strategy based on Signature Tagged Mutagenesis (STM) (1) that allows the identification of bacterial genes required for persistence in the gut. Two major points were developed for this purpose: (i) a system for efficient random mutagenesis in *L. casei* and (ii) a reliable phenotypic screening method to determine the ability of each mutant to maintain in the gut.

A two-plasmid system for transposon mutagenesis, named *Pjunc-TpaseIS*1223 system, was designed (2). A first plasmid, allowing the transient expression of TpaseIS1223, the *Lactobacillus johnsonii* IS1223 transposase gene, was associated with a second suicide transposable plasmid carrying the target sequences of TpaseIS1223. Seventy different tags were cloned in the second, transposable plasmid generating 70 different tagged transposable plasmids. This system gave rise to efficient transposition of tagged transposable plasmid in *L. casei*. A tagged transposon mutant library composed of 60 pools of 70 different mutants was designed so that each tagged mutant of the pool can be quantified by real-time qPCR.

Four pools of 70 tagged-mutants each were challenged in rabbit ileal loop during 16 hours to determine their ability to maintain in the gut. Each pool was injected in loops of two different animals. For each mutant, the quantitative variation between the inoculation pool and the recovered pool was determined thanks to qPCR. Two mutants were shown to be altered in their maintenance. The same mutants were identified in the two rabbits, validating the screening method.

This validated strategy will be carried out to decipher genes involved in probiotics maintenance in gut and could also be used to study bacterial adaptation to other particular environments.

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Populational and individual adaptation of bacteria

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Salmonella typhimurium is an intracellular pathogen that can survive and replicate within host cells. Among the population of intracellular bacteria, their individual contribution to the success of the infection remains largely unknown. The question we addressed was to understand why, among a population of bacterial cells genetically identical, some of them have the ability to proliferate in the host and others don't. The first goal of this work was to study the populational heterogeneity of bacterial cells under infection conditions. In a first set of experiences, we analyzed the ability of Salmonella to grow in LB after various times of infection in the host: Mice macrophages were infected, intracellular bacteria were collected, transferred under a microscope in a 37°C chamber, and their individual viability was monitored either under agar pad (ability to form colonies) or under LB flux (measure of the bacterial elongation). We had shown that three hours post-infection, an important part of the bacterial population lost its ability to grow. Moreover, among the viable cells, their doubling time was extremely variable, suggesting a different metabolic and/or physiological state from one bacterium to the other.

Recently, we used dedicated fusions to characterize the environment in which Salmonella survives and replicates (1, 2). The expression level of each fusion reflects the environment sensed by the bacterium in its vacuole: reactive oxygen species, pH, bile salts, magnesium...

Fusing the promoter of ahpC to the GFP, we measured the level of hydrogen peroxide (H₂O₂) as sensed by intracellular Salmonella and we compared the individual fluorescence level of each bacterium to their ability to elongate. Our first results suggest that the ability of bacterial cells to proliferate is independent of the level of oxidative stress experienced in the host.

Fusing the osmY gene to the GFP, we studied the adaptation of Salmonella to bile (2). Exposure to bile salts induced the expression of the osmY fusion in a consistent manner. Bacterial cells grown in the absence of bile showed lower and more homogeneous fluorescence levels. However, a significant degree of heterogeneity was observed, indicating that some cells activate the fusion in the absence of bile salts. Hence, preadaptation to bile may result from activation of bile resistance responses in a subpopulation of bacterial cells. The latter phenomenon fits in current views indicating that phenotypic heterogeneity is common in clonal bacterial populations, and that gene expression fluctuations can have adaptive value.

References

Involvement of Long Polar Fimbriae in the translocation of Enterohemorrhagic Escherichia coli across the human intestinal barrier

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Enterohemorrhagic Escherichia coli (EHEC) are food-borne pathogens responsible for diarrhea, hemorrhagic colitis (HC) and life-threatening complications such as hemolytic-uremic syndrome (HUS). Translocation of bacteria and Shiga-toxins (Stx) from the gut lumen to underlying tissues is a decisive step in the development of the infection, but mechanisms involved remain unclear. Genomic analysis of the EHEC reference strain EDL 933 has revealed the presence of two lpf operons encoding Long Polar Fimbriae (LPF) previously described in Salmonella Typhimurium and Adherent Invasive E. coli. LPF are adhesins which may play a key role in bacterial targeting to M cells overlying Peyer’s patches (PP). The aims of the study were to analyze the ability of EHEC strains (EDL 933 and isogenic mutants deleted for lpfA1 and/or lpfA2 genes) to (i) interact in vivo with murine PP in ileal loop assays and (ii) translocate in vitro using a M cell model.

We demonstrated that EHEC wild type strain EDL 933 was able to interact with murine PP and translocate across ileal mucosa containing PP while no translocation across ileal biopsies without PP was observed. Interestingly, in competitive colonization assays in ileal loops, lpf isogenic mutants were not able to interact with ileal biopsies containing PP compared to wild type strain, showing that the deletion of one or both lpf operons prevents interaction with murine PP. Moreover, we showed that EHEC strain EDL 933 translocates across M cells at levels significantly higher than those observed for isogenic mutants (EDL 933 ΔlpfA1, EDL 933 ΔlpfA2 and EDL 933 ΔlpfA1-ΔlpfA2).

This study suggests that LPF are involved in the interaction with murine PP and are needed for an active translocation across M cell monolayer. Investigations of virulence factors involved in the interactions of EHEC with M cells could help in designing novel therapeutic approaches against EHEC infection.


Stringent Response and Persistence in *E.coli*.

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Bacteria form persisters, individual cells that are highly tolerant to different types of antibiotics. Persister cells are genetically identical to non-tolerant kin but have entered a dormant state in which they are recalcitrant to the killing activity of the antibiotics. We previously reported a simple model for persister formation according to which mRNases encoded by Toxin-Antitoxin loci (TA) are activated in a small fraction of growing cells by Lon-mediated degradation of the antitoxins (1). Activation of the mRNases, in turn, inhibits global cellular translation and thereby induces dormancy and persistence. The most straight-forward explanation for stochastic persister formation is that Lon activity varies in single cells due to fluctuations of the number in Lon molecules and/or molecules that regulate Lon activity (molecular noise). However, Lon can be activated by polyphosphate (PolyP), a linear polymer of orthophosphate residues (2). In *E. coli*, PolyP is synthesized by polyphosphate kinase (PPK) and degraded by exopolyphosphatase (PPX). Remarkably, The alarmone (p)ppGpp, the central mediator of the stringent response that reprograms the cells to survive nutritional limitations, competitively inhibits PPX and thereby stimulates the accumulation of PolyP (3).

Here we show that both PolyP- and (p)ppGpp-deficient strains exhibit a reduced level of persister cell formation. Additional genetics experiments reveal that the (p)ppGpp level governs bacterial persistence in a PolyP/Lon dependent manner. These results raise the possibility that PolyP accumulation (in response to (p)ppGpp accumulation) programs Lon to degrade the antitoxins. Indeed, proteomic analysis reveals that antitoxins are more stable in both Lon, PolyP- and (p)ppGpp-deficient strains. Together, our results support a model in which (p)ppGpp induces persistence by activating TA loci via PolyP and Lon. Moreover, it is well known that the cellular level of (p)ppGpp varies inversely with the growth-rate. Interestingly, we find that the persistence level of *E. coli* also varies inversely with the growth rate.

Thus, our model provides a simple, testable link between the generation of persisters and the growth-rate and for the first time yields a testable explanation of the common observation that slowly growing bacteria generate higher levels of persisters than rapidly growing ones.

Analysis of the hydrogen photo-production machine in the model cyanobacterium *Synechocystis*


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The hydrogenase enzyme complex (Hox) of the model cyanobacterium *Synechocystis* 6803 has the potential for the sustainable production of hydrogen from nature’s most abundant resources: water and solar energy. The Hox enzyme comprises 5 protein subunits HoxEFUYH, which use various redox centers (Ni-Fe and Fe-S) to catalyze the reaction $2H^+ + 2e^- \leftrightarrow H_2$ with a bias to $H_2$ production. To turn the potential of the Hox enzyme for $H_2$ production into a biotechnological reality, we need to increase both the abundance and the oxygen tolerance of the Hox enzyme ($O_2$, the major product of photosynthesis, inhibits Hox activity). This is what we are doing, taking advantage of our 30-years experience in the genetics and biology of cyanobacteria (http://www.researcherid.com/rid/E-7394-2010). To increase the level of $H_2$ production, we have replaced the weak natural promoter of the *hoxEFUYH* operon with either a strong temperature-controlled promoter (PTR) or a constitutive promoter (PC). After the PCR and DNA sequencing validation of these mutants, we have started their physiological analysis and we found that they grow as healthy as the wild-type strain in the standard photoautotrophic conditions. Then, we have verified that these mutants exhibit a higher level of expression (about 30 fold) of the *hoxEFUYH* operon (quantitative PCR analysis) and of the corresponding HoxF and HoxH proteins (Western blottings analysis with anti-HoxF and anti-HoxH antibodies). We found that the hydrogenase activity of these mutants was increased, three-fold, i.e. less so that the expression of the *hoxEFUYH* genes (30-fold). This finding indicates that we must also increase the abundance of the six protein-subunit Hyp machine (HypABCDEF) which assembles the HoxEFUYH enzyme, as well as the $O_2$ tolerance of the HoxEFUYH enzyme. We will present the complex genetic engineering strategy we designed to (i) increase the production of the HoxEFUYH and HypABCDEF enzymes within the same *Synechocystis* cells; and (ii) mutagenize the HoxH subunit to improve the $O_2$ tolerance of the HoxEFUYH enzyme. We will also report on the transcriptome analysis of the global adaptation of *Synechocystis* to an increased production of hydrogen.

In conclusion: by engineering *Synechocystis* 6803 we are able to enhance the photo-production of hydrogen, and characterize the global adaptation to an increased production of hydrogen.
The *Salmonella* kinase SteC regulates the host actin cytoskeleton by activating MEK via a novel mechanism

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*Salmonella* enterica serovar Typhimurium replicates inside mammalian cells within membrane-bound compartments called Salmonella-containing vacuoles (SCVs). Intracellular replication is dependent on the activities of several effector proteins translocated across the vacuolar membrane by the *Salmonella* pathogenicity island 2 (SPI-2)-type III secretion system (T3SS). We previously reported that the kinase activity of the *Salmonella* SPI-2 T3SS effector SteC induces the formation of a dense meshwork of F-actin around SCVs. In the present work we have used a variety of different approaches to show that SteC promotes reorganization of the actin cytoskeleton by activating a signalling pathway involving MEK, ERK, myosin light chain kinase (MLCK) and Myosin IIB. We found that SteC phosphorylates MEK directly on S200, a previously unstudied phosphorylation site. Molecular dynamics analysis predicts that S200 phosphorylation induces the displacement of a negative regulatory helix causing autophosphorylation on the known MEK activatory residues, S218 and S222. In support of this, phosphomimetic mutations of S200 stimulated phosphorylation of S218 and S222 in host cells. Furthermore, we showed that S200 phosphorylation is important for actin polymerisation mediated by the SteC-MEK pathway. Deletion of steC or its kinase activity led to a modest increase in intracellular replication of *S. Typhimurium* in epithelial cells and macrophages and bacterial growth in mice. This shows that SteC and possibly the actin meshwork act to restrain bacterial growth and thereby regulate virulence.
Characterization of *Yersinia pestis* siderophore secretion inhibitor

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Iron scavenging by the low-molecular-weight iron chelators (siderophores) is a virulence-associated trait of the pathogenic bacteria that are able to produce multiple siderophores active in different conditions. *Y. pestis* (the plague agent) produces a siderophore yersiniabactin (Ybt) encoded by the pgm-locus and induced at 37°C under iron-deficiency [1]. Absence of the siderophore activity in the pgm-strains on the indicator chromogenic chrome azurol S (CAS) agar led to the conclusion that Ybt is the only *Y. pestis* siderophore [2]. However, another siderophore, encoded by the ysu-locus and designated yersiniachelin (Ych), was expressed at 26°C and bound to autoagglutination factor (AF), Hcp-like component of the type six secretion system (T6SS) [3]. Ych was synthesized both by pgm+ and pgm- strains, though the latter arrested it inside the cells and demonstrated a CAS-negative phenotype. The siderophore secretion by the pgm+ strains at 26°C was blocked by the addition of the pgm- cells suggesting that they produce a putative siderophore secretion inhibitor (SSI) which is examined here.

SSI expressed by the pgm- strains at 26°C in the presence of iron was complexed with AF on the cell surface. The AF preparation obtained from the Y.pestis ybt-,ych-strain [3] was used for the SSI purification by ethyl acetate extraction and HPLC. SSI appeared to be a 380.6 Da hydrophobic iron-bound fluorescent compound, which exerted multiple inhibitory effects on the pgm+ strains reducing their autoagglutination, siderophore secretion, and ability to bind the Congo red dye.

SSI blocked the Congo red binding of AF purified from the pgm+ strains and immobilized on nitrocellulose membranes. SSI when injected i.p. into mice, either per se, or in combination with the avirulent TS pgm+ strain, or with the purified AF, stimulated the neutrophil migration to the site of injection. Thus, SSI produced by the attenuated pgm- strains may be regarded as an «antivirulence factor» that not only interferes with the siderophore secretion but also attracts the neutrophils which kill *Y. pestis*. Whether SSI is involved in T6SS regulation because of its association with AF, as well as the mechanism behind its production by *Y. pestis* strains lacking Ybt have to be elucidated.

**CpgA phosphorylation by the Ser/Thr kinase PrkC is crucial for *Bacillus subtilis* growth and morphology.**


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In *B. subtilis*, the ribosome-associated GTPase CpgA is crucial for growth and proper morphology (Campbell *et al.*, 2005; Cladière *et al.*, 2006), and was shown to be phosphorylated *in vitro* by the Ser/Thr protein kinase PrkC (Absalon *et al.*, 2009). It has been shown that PrkC is a key signaling enzyme involved in the exit from dormancy of spores in response to peptidoglycan fragments released by cells in the extracellular milieu (Shah *et al.*, 2008; Squeglia *et al.*, 2011). To further understand the function of CpgA, we first demonstrated that, like its *E. coli* ortholog RsgA, its GTPase activity was stimulated by association with the ribosome, especially with the 30S subunit. The role of CpgA phosphorylation was then analyzed. A single phosphorylated residue, Threonine 166, was identified by mass spectrometry. Using phosphoablative and phosphomimetic CpgA variants, CpgA phosphorylation was found to enhance both its affinity for the 30S ribosome subunit and its GTPase activity. Furthermore, cells expressing a non-phosphorylatable CpgA protein present the same morphological and growth defects as a *cpgA*-deleted strain. Altogether, our results show that CpgA phosphorylation would modulate its ribosome-induced GTPase activity. Knowing the role of PrkC in *B. subtilis* spore germination, we propose that CpgA phosphorylation represents a key regulatory process essential for *B. subtilis* development.

Global regulation of virulence factors of the enteropathogen Yersinia enterocolitica by the RNA chaperone Hfq

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To establish a successful infection, bacterial pathogens need to rapidly coordinate the expression of virulence factors and remodel their surface to colonize the host and escape host immune mechanisms. In addition to transcriptional regulation, bacterial genes are also regulated at the post-transcriptional level by small RNAs (sRNAs) which modulate mRNA stability and translation. The conserved RNA chaperone Hfq mediates the interaction of many sRNAs with their target mRNA(s), thereby playing a global role in protein expression.

As a model for an extracellular enteropathogen, we study the Gram-negative bacterium Yersinia enterocolitica. Following ingestion of contaminated food or water, bacteria reach the terminal ileum, adhere to and subsequently penetrate the mucosal epithelium, and multiply extracellularly in the lymphoid tissue. We investigated the significance of Hfq for the global regulation of virulence factors using a proteomic approach as well as immunodetection and electron microscopy. Our functional analysis showed that Hfq facilitates the expression of early virulence genes such as urease, which allows bacterial survival after ingestion and passage through the acidic stomach, and the adhesin invasin which is essential for invasion through the gut epithelial barrier. Hfq also promoted expression of proteins produced later in infection such as the adhesin YadA, an essential virulence factor important for attachment to host cells and serum resistance. In contrast, Hfq negatively regulated the expression of several surface associated proteins, including the Myf fimbriae, and two proteins of the Ail family (Ail and OmpX). In Y. enterocolitica, Ail is involved in serum resistance, attachment to and invasion of host cells. All phenotypes could be complemented by providing hfq on a plasmid.

Although Hfq appears to be a global regulator of many virulence factors, it was dispensable for protein secretion by the type III secretion system of Y. enterocolitica, unlike what was described for other pathogenic bacteria.

Taken together our results indicate that Hfq promotes the coordinate expression of virulence factors and is particularly important in remodeling the bacterial surface of Y. enterocolitica. Identification and characterization of Hfq-dependent sRNAs which mediate this global regulation are currently under way.
RNP profiling: a multidimensional approach to explore RNA-protein interactions at the global level


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Over the last decade, bacterial RNA biology has much focused on regulatory RNA-RNA interactions, especially modulation of mRNA translation and stability by small non-coding RNAs (sRNAs). However, to what extent RNA molecules act in concert with or directly influence activity or localization of cellular proteins is much less understood. Essentially, all functions of RNA molecules in the cell including transcription, translation, processing and modification of macromolecules involve formation of RNA complexes with proteins. These interactions, be they transient or stable, represent particular aspects of the molecular “behavior “ of RNA and may constitute the minimal structural-functional units with direct implications in their physiological functions. Thus, we must determine the global landscape of RNA-protein associations to understand how RNA functions are organized in the cell.

Here we introduce a novel RNP profiling approach wherein a classical biochemical protocol, the density gradient sedimentation, is combined with two high-throughput methods to explore in an unbiased way the RNA-protein interactome of the model pathogen Salmonella Typhimurium. The glycerol gradient sedimentation allowed efficient resolution of all soluble macromolecular complexes up to the size of ribosome. Total RNA and protein were isolated fraction-wise and subjected to RNA-seq and global mass-spectrometric peptide counting, respectively. After normalization we could recapitulate in-gradient distributions for >4000 individual RNAs and >2000 proteins. Extensive proof-of-principle analysis, including Western and Northern blots of several well-characterized complexes as reliable alternative semi-quantitative methods, confirmed the high precision and reproducibility of observed sedimentation patterns and intermolecular associations. We have also detected a wealth of unusual distribution profiles indicating that up to 20% of all Salmonella RNAs do not conform to known generic patterns and may represent new RNA-protein complexes of novel functions.
Bacterial persisters are non-growing cells that are tolerant to antibiotic treatment. They have been studied mainly in Escherichia coli in vitro and are reported to form stochastically at very low frequency. All bacterial species form persisters and they are particularly relevant in the context of chronic infections that are recalcitrant to antibiotic treatment. Salmonella enterica causes acute and chronic infections by replicating and surviving for long periods of time within host cells, notably macrophages. Using a dual fluorescence reporter method, Fluorescence Dilution (FD), we reported previously that a large proportion of the intra-macrophage population of Salmonella is non-replicating. Here we show that even brief internalization by macrophages hugely enhances the formation of Salmonella persisters, suggesting the existence of a signal for their formation. Intracellular persisters appear to form a heterogeneous population and to be in a different physiological state to those generated in vitro. Finally, we report that long-term intracellular persisters can reinitiate intracellular growth after reinfection of macrophages. Together these data provide evidence suggesting that intracellular persisters constitute a reservoir for relapse of infection.
Virus cell-to-cell transmission through “viral biofilms”

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During the past two decades, important aspects concerning the way that viruses spread from cell to cell were discovered. Several types of cell–cell contacts formed between infected cells and target cells, through which viruses could spread, were reported. However, the nature of the infectious material transferred between cells and its organization at cell–cell contacts remained poorly documented.

Our recent finding that the human T-cell leukemia virus type 1 (HTLV-1) encases itself in a carbohydrate-rich adhesive extracellular ‘cocoon’, which enables its efficient and protected transfer between cells, unveiled a new infectious entity and a novel mechanism of viral transmission. These HTLV-1 structures are observed at the surface of T cells from HTLV-1-infected patients and are reminiscent of bacterial biofilms. The virus controls the synthesis of the matrix, which surrounds the virions and attaches them to the T cell surface. Importantly, these extracellular viral assemblies were key to HTLV-1 spread from cell to cell; treating HTLV-1-infected cells with agents competing with extracellular matrix interactions disrupted HTLV-1 assemblies, strongly inhibiting the capacity of virus-producing cells to infect other cells. When detached from the infected cell, viral clusters and released viral particles were far less infectious.

A tightly regulated balance between adhesion, cohesiveness and dispersion might ensure the equilibrium between viral biofilm generation and dissemination to other cells. Modulation of extra cellular matrix composition by viral-genome expression in infected cells could be a key feature for viral spread. Whether the production of the viral biofilm could be an antiviral mechanism developed by the infected cell to encase infectious viral particles, which has been hijacked by HTLV-1 to spread efficiently remains to be determined.

The finding of extracellular viral assemblies with the structural and functional features of biofilms opens novel perspectives for the understanding of virus dissemination. We propose that, similar to bacterial biofilms, viral biofilms could represent ‘viral communities’ with enhanced infectious capacity and improved spread compared with ‘free’ viral particles, and might constitute a key reservoir for chronic infections.
The blue light-sensitive YcgF-YcgE pathway in *Escherichia coli*

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*Escherichia coli*, which can switch between the environmental and host-associated lifestyles, expresses the photoreceptor protein YcgF. The N-terminal BLUF domain of YcgF is responsible for sensing blue light using the flavin chromophore FAD and is associated with a C-terminal EAL-domain (1). In general EAL domain-containing proteins are known to act as phosphodiesterases (PDE) that degrade the biofilm-promoting second messenger c-di-GMP (2). However, the degenerate EAL domain protein YcgF does not hydrolyse nor bind c-di-GMP but directly interacts and antagonizes the repressor protein YcgE (3). Re-introducing all amino acids typically required for PDE activity did not restore the enzymatic activity or c-di-GMP binding ability of YcgF, but compromised binding to YcgE, indicating that YcgF is not just a defective PDE but has structurally evolved to bind YcgE.

YcgE is a MerR-like protein and represents a closely related parologue of MlrA. MlrA is a transcription factor that cooperates with an active PDE to control the transcription of the curli regulatory gene *csgD* (2, 4). Our recent footprint analysis showed that YcgE and MlrA recognize similar operator binding sites. Gel retardation as well as protein-protein interaction studies revealed that the YcgF/YcgE-system, when overproduced, can still cross-talk into the MlrA/curli-system, reflecting the common evolutionary origin.

The blue-light regulated binding of YcgF to YcgE induces a release of YcgE from its operator located upstream of the *ycgZ-ymgABC* operon, which leads to derepression of these genes which play a role in biofilm maturation (3). By a hitherto unknown mechanism YmgA and YmgB activate colanic acid production and downregulate curli fimbriae synthesis via the complex two component system RcsBDC (3). Our initial bacterial-two-hybrid analysis indicates that these small proteins under YcgF/YcgE control interact in a complex that may directly contact the RcsBDC signal transduction system.

A modular molecular motor drives both gliding motility and sporulation in *Myxococcus xanthus*

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How gliding motility on solid surfaces is achieved in *Myxococcus xanthus* has long remained enigmatic, mostly because movement does not involve obvious extracellular organelles. Recently, we demonstrated that motility in *M. xanthus* is driven by a proton channel composed of one MotA homolog (AglR) and two MotB homologs (AglQ and AglS). This motor cooperates with the bacterial actin cytoskeleton to transport an envelope-spanning Glt motility protein complexes at the cell surface directionally. Motility is produced as a motility machinery surface tip-bound polysaccharide (slime) acts like a glue to immobilize the transported Glt complexes against the substratum.

In the course of this study, we also made the surprising discovery that the AglRQS motor is essential not only for motility but also for sporulation, a cellular process during which the cells become surrounded by a thick polysaccharide (the spore coat) that confers resistance during unfavourable environmental conditions. We identified the mechanism and demonstrated a direct interaction between the AglRQS motor and a previously identified Nfs envelope complex, a close homolog of the Glt complex. Transmission electron microscopy, time-lapse microscopy and localization studies on sporulating cells, showed that the AglRQS motor rotates the Nfs complex directionally around the spore cortex, thus laying a compact and dense spore coat around the future spore.

These results highlight the existence of new class of bacterial motors involved in intracellular transport of sugar-associated complex. These modular motors can be adapted to specific functions based which output complex they interact with. In the future, we hope to understand how these motors specifically interact with different transducers to determine the extent of the physiological processes driven by these nanomachines in bacterial cells.
Effect of oral administration of lactobacilli on orally acquired listeriosis.
C Archambaud\textsuperscript{a,b,c}, MA Nahori\textsuperscript{a,b,c}, G Soubigou\textsuperscript{d}, C Bécavin\textsuperscript{a,b,c}, P Lechat\textsuperscript{e}, T Smokvina\textsuperscript{f}, P Langella\textsuperscript{g}, M Lecuit\textsuperscript{h,i,j} and P Cossart\textsuperscript{a,b,c}

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The intestinal microbiota has a critical impact on intestinal physiology \textsuperscript{1}. Enteropathogens induce an alteration of the gut homeostasis that might be modulated by commensals and/or by probiotics. Here we have investigated the impact of two \textit{Lactobacillus} species, \textit{Lactobacillus paracasei} and \textit{L. casei}, on \textit{Listeria monocytogenes} (\textit{Lm}) and in a gnotobiotic humanized mouse model of orally acquired listeriosis. \textit{Lm} is a foodborne pathogen that crosses the intestinal barrier and disseminates within the host \textsuperscript{2}. This work took advantage of a series of tools we have developed, including \textit{Lm} specific tiling arrays \textsuperscript{3} and a knock-in mouse expressing a humanized version E-cadherin (E16P) \textsuperscript{4} permissive to orally-acquired listeriosis and reproducing the human disease. We first show that exposure to each \textit{Lactobacillus} species decreases \textit{Lm} systemic dissemination in orally inoculated mice. A whole genome intestinal host transcriptomic analysis revealed that each \textit{Lactobacillus} changes expression of a specific subset of genes during infection, with interferon-stimulated genes (ISG) being the most affected by both lactobacilli. We also examined host microRNAs (miRs) expression and identified three miRs repressed during \textit{Lm} infection whose level was increased by the \textit{Lactobacillus} exposure. Finally, we showed that \textit{Lactobacillus} significantly reshapes \textit{Lm} transcriptome and in particular up-regulate genes encoding enzymes allowing \textit{Lm} utilization of intestinal carbon and nitrogen sources. Altogether, this comprehensive analysis reveals that the impact on \textit{Lm} infection after exposure with lactobacilli correlates with a decrease in host genes expression, in particular ISGs, miRs regulation and an important reshaping of \textit{Lm} transcriptome.

New mechanisms for “host iron” acquisition in Bacillus cereus


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Bacillus cereus is a Gram-positive bacterium, opportunistic pathogen for human, frequently associated with food-borne infection. The ability of B. cereus to colonize the host (mammal or insect) is linked to the presence of several adaptation factors, one of which is the capacity to acquire iron. Previously, an in vivo screen of B. cereus led to the identification of a novel protein, IlsA (Iron-regulated leucine rich surface protein), which is specifically expressed in vivo in the insect hemocoel and under iron restrictive conditions in vitro (Mol. Microbiol 62:339-55, 2006). It was further shown that IlsA is localized on the surface of B. cereus and affinity tests revealed that IlsA interacts with both hemoglobin, (probably due to the presence of a NEAT domain) and host ferritin. Inactivation of ilsA decreases the ability of B. cereus to grow in the presence of especially ferritin indicating that IlsA plays a role in iron acquisition from this iron source. In addition, the ilsA mutant displays reduction in growth and virulence in an insect model Galleria mellonella (PloS Pathogens, 2009 (11) e1000675). In order to further analyze how IlsA takes part in iron acquisition from hemoglobin and ferritin we are actually searching for possible partners playing a role in transport and iron release. The role of Isd components, (Iron surface determinants) previously identified in B. anthracis and S. aureus as being involved in iron uptake from heme, are considered. To understand how iron is released from host ferritin and transported into the bacterial cells, investigations on the interaction between IlsA and ferritins and on the roles of the B. cereus siderophores (bacillibactin and petrobactin) have been done using reverse genetic and biophysical techniques. Our data suggest that IlsA may contribute to unfold the ferritin shell, which will accelerate iron acquisition by the bacillibactin. This finding reveals for the first time the mechanisms of host ferritin use by bacteria and highlights the interplay between surface proteins and siderophores. In conclusion, we provide insights into the host adaptation of B. cereus, IlsA being a unique key factor of iron acquisition, specifically adapted for interaction with several iron-rich host proteins found in vertebrates and invertebrates.