5 / Microbial Biotechnology

Research in the Department of Microbial Biotechnology focusses on microbes of environmental, industrial or clinical relevance. Our work includes several approaches based on molecular genetics, systems and synthetic biology, genomics, proteomics and metagenomics. The scientific objectives of the department include five complementary aspects of microbial biology:

• Environmental microbiology. We study the regulatory mechanisms that degrade organic pollutants by analysing global regulation networks that control the hierarchical assimilation of nutrients in complex environments. Understanding the overall regulation of bacterial metabolism will allow us to optimise bioremediation strategies and industrially important biotransformation processes. We use metagenomic approaches to evaluate the effect of toxic compounds, including biocides and herbicides, on natural microbial communities.

• Microbial pathogens. We direct our efforts to host-pathogen interactions in infections caused by intracellular and opportunistic bacterial pathogens. In addition, we study basic processes of microbial physiology, such as cell division, that are relevant in infection and to define antimicrobial targets.

• Microbial resistance to antibiotics and the search for new antimicrobials. We work to understand the mechanisms of bacterial resistance to antibiotics and to analyse the complex responses elicited upon exposure of microbes to sub-lethal concentrations of antibiotics. In addition, we search for new targets as a way to develop new antimicrobials.

• Microbial responses to hostile environments. The purpose is to determine bacterial responses to stressful environments, including general stress responses and specific responses to agents that cause DNA damage. We study how bacterial viruses and yeasts replicate their DNA and how bacteria repair DNA damage and promote segregation to improve genome stability.

• Microbial engineering. The goal is to generate bacterial strains optimised to obtain products of interest (recombinant antibodies, hydrolytic enzymes), or to detect and degrade pollutants. We engineer bacterial strains that attach to specific surfaces such as antigen-expressing human cells, including tumour cells, which often express proteins abnormally on the plasma membrane.

Genetic stability

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Our research centres on the study of the molecular mechanisms that bacteria of the Firmicutes phylum use to maintain genomic stability, promote horizontal gene transfer and achieve accurate plasmid segregation. Using *Bacillus subtilis* as a model, we showed that the DNA damage response recruits various complex molecular machineries depending on the type of DNA damage, double-strand breaks (DSB) or single-strand gaps (SSG). We found that RecN, in concert with PNPase, promotes dynamic recruitment of DNA ends onto a repair centre. The AddAB or the RecJ-RecQ(RecS)-SsbA complex creates a 3'-ssDNA tailed duplex at the breaks, and RecN recruits RecA mediators (SsbA, RecOR) and modulators (RecF, RecX, RecU, RecD) to initiate recombinational repair (Fig. 1). In addition, we observed that DisA, in concert with RadA/Sms and c-di-AMP, recognise recombination intermediates and modulate SSG repair. More than 150 genes are switched on during natural competence, with DprA, SsbB, SsbA, RecO(R), RecX, and CoiA contributing to RecA activation to increase genetic diversity. By studying the segregation machineries, we showed that i. low copy number plasmids require homodimeric ParA-like (δ2), ParB-like (ω_2) and *parS* regions for stable inheritance at cell division (Fig. 2); ii. δ_2-ATP, which associates dynamically to the nucleoid, captures and tethers the plasmid-bound ω_2-*parS* complex to the nucleoid; iii. at stoichiometric δ_2 and ω_2 concentrations, the latter facilitates ATP hydrolysis, creating a gradient of nucleoid-bound self-organising δ_2 clouds; and iv. dynamic assembly/disassembly of the nsDNA-δ_2-ω_2-*parS* complex moves the plasmid molecule towards the cell poles to guarantee faithful segregation.

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Yadav T, Carrasco B, Hejna J, Suzuki Y, Takeyasu K, Alonso JC. J Biol Chem 2013; 288:22437-22450

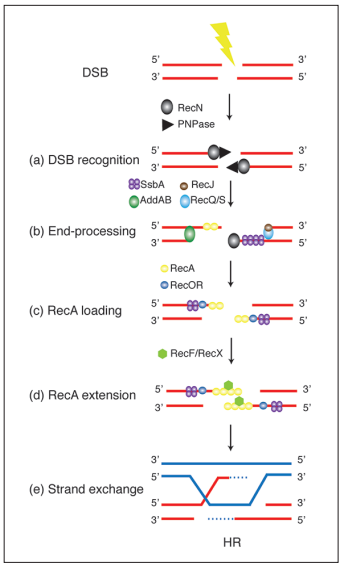
Vlasic I, Mertens R, Seco EM, Carrasco B, Ayora S, Reitz G, Commichau FM, Alonso JC, Moeller R. *Bacillus subtilis* RecA and its accessory factors, RecF, RecO, RecR and RecX, are required for spore resistance to DNA double-strand break. Nucleic Acids Res 2014; 42:2295-2230

Quiles-Puchalt N, Carpena N, Alonso JC, Novick RP, Marina A, Penadés JR. Staphylococcal pathogenicity island DNA packaging system involving cos-site packaging and phage-encoded HNH endonucleases. Proc Natl Acad Sci USA 2014; 111:6016-6021

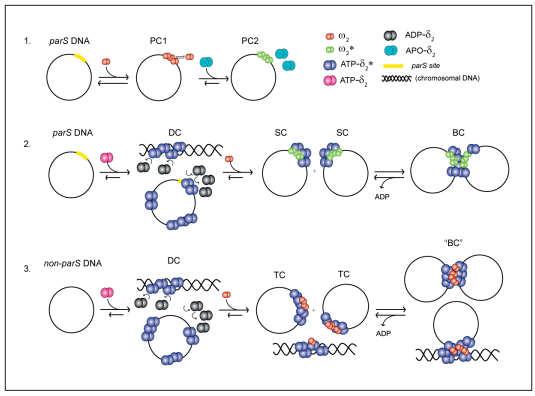
Yadav T, Carrasco B, Serrano E, Alonso JC. Roles of *Bacillus subtilis* DprA and SsbA in RecA-mediated genetic recombination. J Biol Chem 2014; 289:27640-27652

PATENT

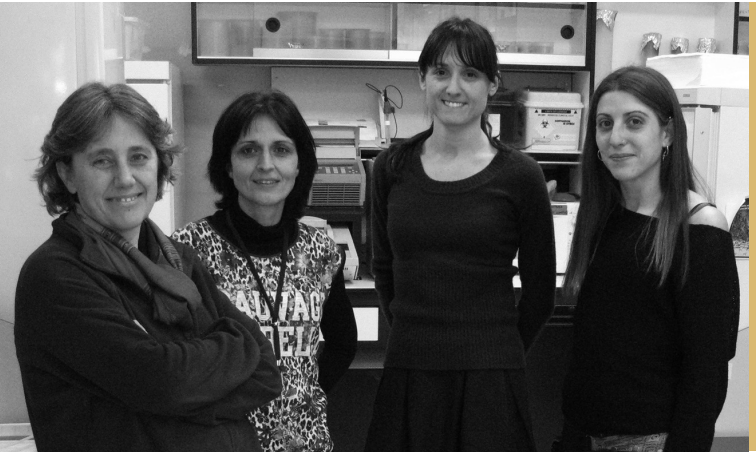
20140308246. A method of making biological containment factors for use in selectively killing target bacteria such as *Bacillus anthracis*, *Clostridium botulinum*, *Clostridium perfringens*, and other select agents



1 *Early stages in DNA repair by homologous recombination in B. subtilis. RecN, in concert with PNPase and SsbA, binds ssDNA regions at broken DNA ends (DSB recognition). Long-range end processing catalysed by the AddAB or the RecJ-RecQ(RecS)-SsbA complex generates a 3'-ssDNA tail coated by SsbA (end-processing). RecN might recruit the RecO-RecR complex there. RecO promotes SsbA disassembly and loads RecA onto ssDNA (RecA loading). A RecA filament at one of the processed ends promotes invasion of an intact homologous DNA to form the displacement loop (D-loop) recombination intermediate modulated by RecF and RecX.*



2 *Dynamic assembly of different types of protein-DNA complexes. 1. Protein ω_2 binds parS DNA to form an unstable partition complex PC1, but after interaction with Apo-δ_2, it undergoes a conformational transition leading to ω_2* and stable PC2. 2. δ_2 interaction with any DNA induces conformational changes in the protein (ATP-δ_2*). Protein δ_2 binds DNA and forms dynamic complexes (DC). Protein ω_2* at the stable PC2 promotes δ_2 redistribution to the segregosome (SC). Two or more SC produce a large higher-order complex (BC). δ_2 at parS regulates SC and bridging complex (BC) formation. 3. Interaction of δ_2 at the DC with ω_2 facilitates TC formation and accumulation of "BC".*



Recombination-dependent DNA replication

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Our research centres on the mechanisms that cells use to continue DNA replication when this process encounters impediments that can collapse the fork, producing a broken DNA end. Replication restart is then mediated by proteins that were initially identified by their roles in homologous recombination and repair of DNA double-strand breaks (DSB). We use a simple model system, *Bacillus subtilis* and its bacteriophage SPP1, and several biophysical, structural and molecular biology techniques to study the recombination mechanisms that lead to replication restart.

A central step in the recombination reaction is the resolution of the recombination intermediate. Using single molecule analysis, we studied the cleavage preference of the RecU Holliday junction-resolving enzyme, and how it is modulated by other proteins such as the branch migration helicases RecG and RuvAB (Figure 1). Our results can be extended to other systems and the method developed should be applicable for the study of mechanisms used by other junction-binding enzymes involved in branch migration and junction resolution of the recombination intermediate.

We have reconstituted the replisomes of phage SPP1 and its host *B. subtilis in vitro*, and analysed the effect of recombination proteins in DNA replication *in vitro* (Figure 2). RecA slightly affects DNA replication *in vitro,* and RecO addition facilitates RecA-mediated inhibition of DNA synthesis. These results will be further analysed in the next few years. They suggest that RecA might prevent potentially dangerous forms of DNA repair that occur during replication.

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Cañas C, Suzuki Y, Marchisone C, Carrasco B, Freire-Benéitez V, Takeyasu K, Alonso JC, Ayora S. Interaction of branch migration translocases with the Holliday junction-resolving enzyme and their implications in Holliday junction resolution. J Biol Chem 2014; 289:17634-17646

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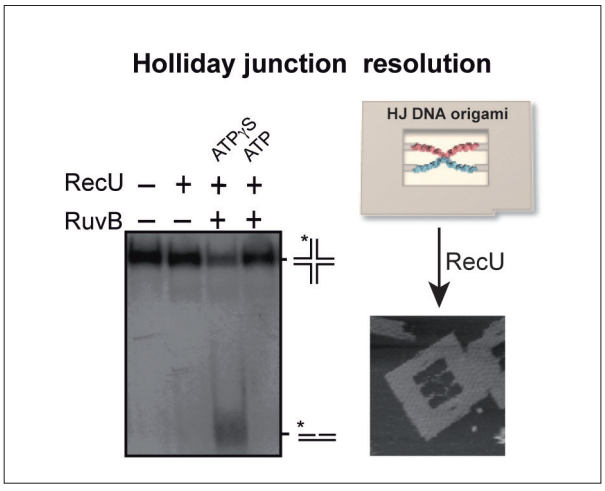

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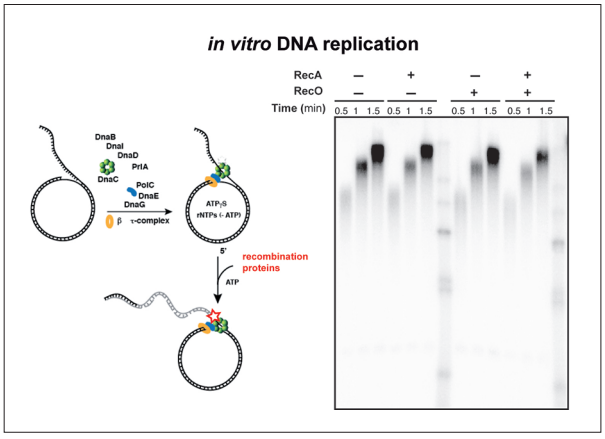

1 *Resolution of the Holliday junction recombination intermediate is modulated by the RuvB protein (left) and can be analysed with the DNA origami technique (right).*

2 *Scheme of the in vitro DNA replication system used (left) and the effect of RecA and RecO recombination proteins on DNA replication (right). Reactions were performed with 13 purified replication proteins from B. subtilis. Purified RecA and RecO recombination proteins inhibited the reaction.*

Stress and bacterial evolution

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The major interest of our group is to understand the bacterial responses to stress. We specifically study hypermutation and hyper-recombination as "bacterial strategies" to speed adaptation to environmental stress. One model used is antibiotic stress and the development of antibiotic resistance. Our work focusses on stable and inducible hypermutation/hyper-recombination in *Escherichia coli, Pseudomonas aeruginosa* and *Mycobacterium smegmatis/tuberculosis.*

We currently study

1. Compensation of stable hypermutation. Once adapted, hypermutable bacteria must decrease (compensate) the high mutation rate to avoid accumulation of deleterious mutations. Our study aims to determine the molecular mechanisms involved in this compensation.

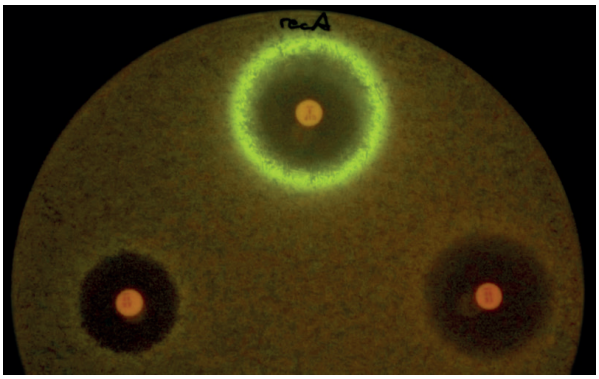
2. Regulation of stress responses and inducible hypermutation.

- Environmental regulation of mutagenesis
- Transcriptional regulation of specialised DNA polymerases (of the SOS regulon)
- Effect of antibiotics on mutation and recombination: do antibiotics promote antibiotic resistance?

3. Hypermutation in bacteria lacking a DNA mismatch repair system (MMR) such as Mycobacterium and Streptomyces. This will allow the use of hypermutant/hyper-recombinant bacteria of industrial interest as biotechnological tools to produce modified biosynthetic pathways.

4. Evolution of resistance to beta-lactam antibiotics and the development of new beta-lactamase inhibitors.

5. The molecular basis of bacterial evolution. Combatting antibiotic resistance by preventing evolution (mutation, recombination and horizontal transfer).



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Cell cycle, DNA replication and genome stability in eukaryotes

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TECHNICIAN:
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SELECTED PUBLICATIONS

Saugar I, Vázquez MV, Gallo-Fernández M, Ortiz-Bazán M., Segurado M, Calzada A, Tercero JA. Temporal regulation of the Mus81-Mms4 endonuclease ensures cell survival under conditions of DNA damage. Nucleic Acids Res 2013; 41:8943-8958

Ayuda-Durán P, Devesa F, Gomes F, Sequeira-Mendes J, Avila-Zarza C, Gómez M, Calzada A. The CDK regulators Cdh1 and Sic1 promote efficient usage of DNA replication origins to prevent chromosomal instability at a chromosome arm. Nucleic Acids Res 2014, 42:7057–7068

We aim to understand the factors and mechanisms by which the cell division programme maintains stable eukaryotic genomes across generations. The complex structure, size, and fragmentation of eukaryotic genomes, and the strict synchrony between termination of DNA replication and initiation of chromosome segregation during cell cycles complicate the transmission of exact genomes to progeny. This complexity and the vast numbers of divisions necessary to reach and maintain cell populations provide ample opportunities for DNA errors. Abortive cell division or aberrant genomes are very infrequent in normal cells; this indicates the robustness of the mechanisms and regulation of cell division programmes, which are yet not fully understood. Although they are rare, undetected errors ensue spontaneously in progeny and can threaten genome stability and health of the organism. Deregulation of proliferation control during the G1 phase, abnormalities during DNA replication, basal activation of DNA damage repair pathways, and unscheduled chromosome segregation are frequently associated with elevated genome instability, appear in most oncogenic cell cycles, and are hallmarks of cancer. The mechanics of genome instability acquisition in deregulated cell cycles at the molecular level is nonetheless poorly understood.

We studied the regulation of pausing of replication forks during DNA replication at fork-pausing sites. This pausing depends on the fork protection complex proteins Tof1/Csm3, which bind to the replisome. We found that Tof1 is regulated during normal S phases by phosphorylation dependent on the essential S phase kinase Cdc7/Dbf4 and on the Mec1 kinase (Figure 1), revealing active control of fork-pausing during DNA replication. We also studied the molecular abnormalities of DNA replication that potentially contribute to genome instability in cells deregulated in the G1 phase. We demonstrated that the CDK inactivators Cdh1-APC/C and Sic1 cooperate to promote efficient activity of DNA replication origins and optimal distribution of replication initiation events on the chromosomes (Figure 2). We also found that this control is essential to maintain low rates of genome instability.

Our experience in analysing chromosome integrity also allowed us to assist other groups in characterising telomere stability and DNA damage repair during DNA replication.

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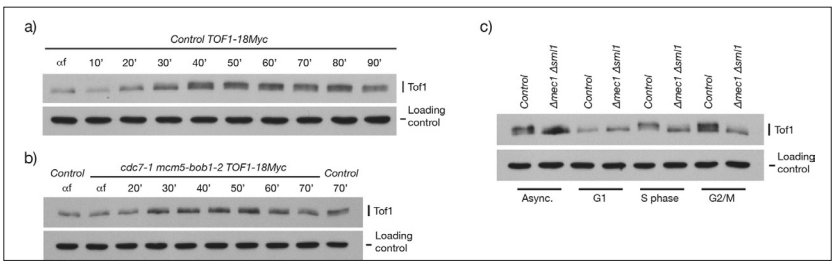

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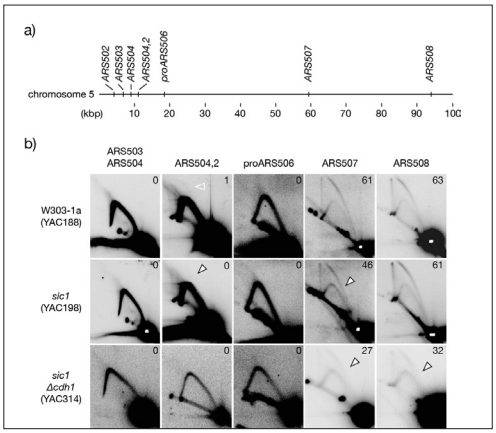

1 *Phosphorylation status of Tof1 during the cell cycle in Saccharomyces cerevisiae cells. a) Control cells. b) Cells lacking Cdc/Dbf4 kinase activity. c) Cells lacking Mec1.*

2 *Two-dimensional electrophoresis of DNA replication intermediates shows that deregulated G1 phase proliferation control impairs the activity of DNA replication origins (arrowheads, decrease in replication bubbles) on a chromosome arm.*

Biotechnology of protein secretion systems in *Escherichia coli*

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Our work focusses on engineering *Escherichia coli* bacteria and their protein secretion systems for biomedical applications. We study protein secretion systems found in enteropathogenic (EPEC), enterohemorrhagic (EHEC) and uropathogenic (UPEC) *E. coli* strains and engineer them to develop new protein expression tools that can be applied for selection, production and *in vivo* delivery of therapeutic proteins, such as recombinant antibodies, by non-pathogenic *E. coli* strains. We work with single-domain antibodies (sdAb) or nanobodies, the smallest antibody fragments with full antigen-binding capacity known to date. Nanobodies are based on single VH domains obtained from heavy chain-only antibodies found in camelids (*e.g.*, dromedaries, llamas). Nanobodies have high affinity and specificity for their antigens and closely resemble human VH sequences.

In the last two years, our studies have concentrated on

1) Developing *E. coli* display technology based on type V secretion systems (T5SS). T5SS members are proteins with the capacity for "self-translocation" across the bacterial outer membrane, like the intimin-invasin family and the classical autotransporters. We have engineered the T5SS translocator domains to display nanobodies on the surface of *E. coli* and selected high-affinity binders of antigens relevant to human disease.

2) Re-programming *E. coli* adhesion to tumours with synthetic adhesins. Nanobody display on the *E. coli* allowed us to generate "synthetic adhesins" that can drive attachment of bacteria to target antigenic surfaces, including tumour cells that express cell surface antigens. We demonstrated that specific tumours can be targeted and colonised efficiently *in vivo* by low doses of engineered *E. coli* strains expressing synthetic adhesins that bind antigens expressed on the tumour cell surface. We intend to develop engineered *E. coli* strains with synthetic adhesins for the early diagnosis and therapy of specific human tumours.

3) Injection of nanobodies from *E. coli* into human cells. We are exploiting the type III protein secretion system (T3SS) from EPEC and EHEC *E. coli* strains for direct delivery of therapeutic proteins and nanobodies from *E. coli* cells into the cytosol of human cells.

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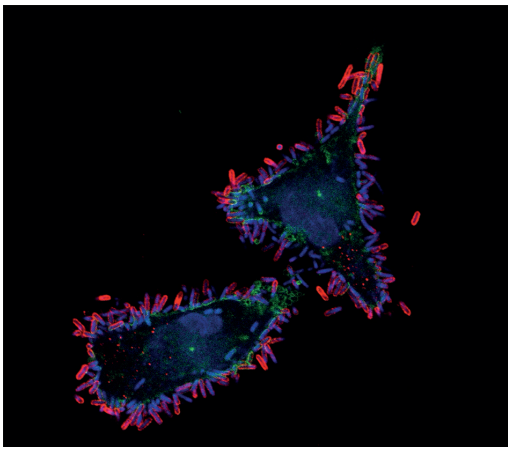

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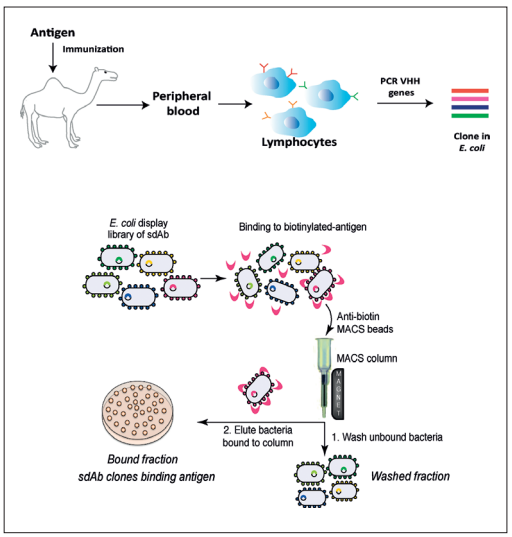

1 *Fluorescence confocal microscopy image showing E. coli bacteria (red) with synthetic adhesins targeting an antigen (green) expressed on the surface of human tumour cells (nuclei and bacterial DNA stained in blue). Synthetic adhesins can drive adhesion of E. coli bacteria to specific cells and surfaces.*

2 *The process of selection of single-domain antibodies (sdAb) by E. coli display. The VHHs gene segments are amplified by the lymphocytes of an immunised dromedary and cloned in a vector with the T5SS translocator domain for expression on the E. coli bacterial cell surface. These bacteria are incubated with the biotin-labelled target antigen, followed by anti-biotin magnetic beads. Antigen-binding clones are captured in magnetic cell sorting (MACS) columns and plated for amplification.*

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PATENT

US 8,623349 B2. System methods and microorganisms for antibody production with type III secretion system

Laboratory of intracellular bacterial pathogens

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Our group studies two "model" intracellular bacterial pathogens important in human and animal health, *Listeria monocytogenes* and *Salmonella enterica* serovar Typhimurium. These bacteria have different cell wall architecture and adopt distinct lifestyles (cytosol versus intra-vacuolar) within the eukaryotic cell. The common aim of our studies is to identify envelope changes during host cell colonisation and to understand how these changes shape the course of infection.

Listeria monocytogenes. This pathogen produces a large number of surface proteins, many exclusive to this bacterium and with unknown function. A family of surface proteins of particular interest to us is that composed of proteins covalently bound to the peptidoglycan after processing of their LPXTG-sorting motif. We have linked the biological function of two *L. monocytogenes* LPXTG surface proteins, Lmo0412 and Lmo1413, to bacteria interaction with the host. Lack of either protein alters virulence in the mouse model. In addition, we dissected the mechanism used by intracellular *L. monocytogenes* to upregulate the LPXTG protein Lmo0514 inside host cells. This mechanism involves the regulatory small RNA (sRNA) Rli27 and a long 5'-UTR present in a defined *lmo0514* mRNA transcript isoform. We are currently pursuing the biological role of Lmo0514 inside host cells.

S. enterica serovar Typhimurium. This pathogen establishes persistent infections in which bacteria reside in a "dormant-like" state within eukaryotic cells. The molecular basis of this phenomenon is poorly understood. Using the fibroblast *S. enterica* Typhimurium infection model, we characterised the expression of numerous regulatory sRNAs in non-proliferating intracellular bacteria, and identified an sRNA species induced in these conditions. We also examined the role of toxin-antitoxin (TA) modules, which respond to many stresses, in establishing persistent infections inside eukaryotic cells. These studies showed an unexpectedly large number of TA modules in *S. enterica* Typhimurium and the selective use of some in distinct host cell types. We continued our studies on the enzymatic machinery that supports peptidoglycan metabolism in intracellular bacteria and those focussed on peptidoglycan sensing during persistent infection. Lastly, we have linked *S.* Typhimurium persistence to a type of autophagy not previously reported in bacterial infections.

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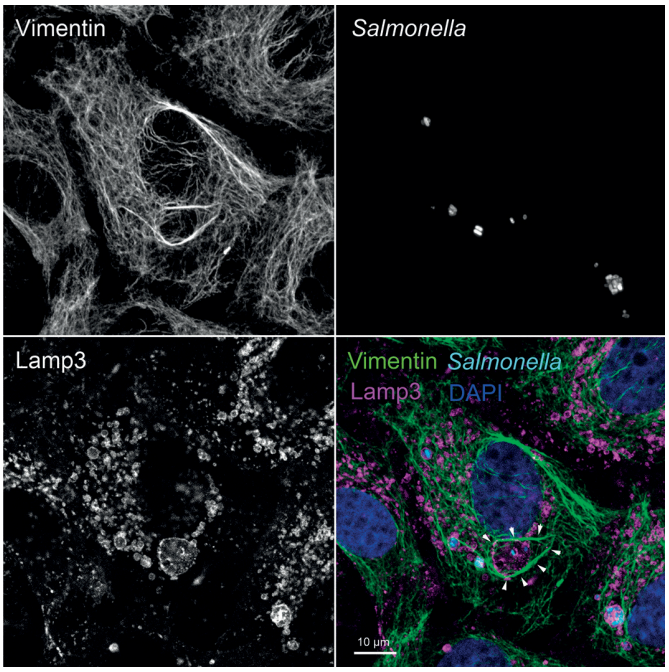
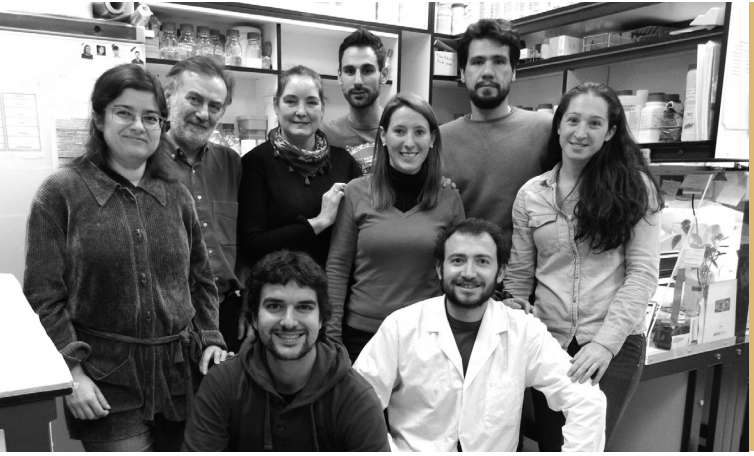


1 *Persistent infection of fibroblasts by S. Typhimurium correlates with an autophagy process that eliminates part of the intracellular bacterial population. NRK-49F rat fibroblasts infected with S. Typhimurium. Note the vimentin cage that segregates the autophagosome that is digesting some bacteria from the remaining cell contents (arrowheads). Other intracellular bacteria persist outside the autophagosome within Lamp3-enriched vacuolar compartments. Vimentin (green), S. Typhimurium (Salmonella, cyan), lysosomal membrane glycoprotein Lamp3 (magenta), nuclei (DAPI, blue). Bar = 10 µm.*



Opportunistic pathogens

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Nosocomial infections due to opportunistic pathogens are a serious health problem. As models for understanding the pathogenetic mechanisms of these microorganisms, we use *Pseudomonas aeruginosa* and *Stenotrophomonas maltophilia*, free-living bacteria with a characteristic phenotype of intrinsic resistance to antibiotics. Acquisition of resistance in these organisms is the result of millions of years of evolution in natural, non-clinical ecosystems, long before humans began to use antibiotics for therapy. We recently expanded our interest to the study of *Klebsiella pneumoniae*.

We study the biology of opportunistic pathogens, focussing on the networks and the evolutionary processes that connect resistance and virulence. This includes analysis of the role of non-clinical natural ecosystems in acquisition and evolution of antibiotic resistance and virulence, and the host responses triggered by these pathogens. We are examining the intrinsic resistome, that is, the set of genes that contributes to the characteristic phenotype of susceptibility to antibiotics of a given bacterial species. Our work could provide information on potential drug targets for use in reducing antibiotic resistance.

We are especially interested in multidrug efflux pumps. These elements are found in all living beings and contribute to many processes, including resistance to anticancer chemotherapy in humans and antibiotic resistance in bacteria. Whereas these pumps can expel these drugs, however, they have different original functions in nature. We recently showed that the efflux pump SmeDEF in *S. maltophilia* is major determinant of resistance to quinolones (a family of synthetic antimicrobials), whose function is colonisation of roots in plants.

Acquisition of resistance can have a fitness cost, reflected as less competitivity of resistant compared with susceptible bacteria. We showed that fitness costs are gene-specific and that the effect on bacterial physiology of acquiring resistance is also specific, rather than a general metabolic burden. We found that fitness costs can be compensated, with no need for secondary mutation, by metabolic rewiring that adjusts bacterial physiology to the changes produced by resistance acquisition.

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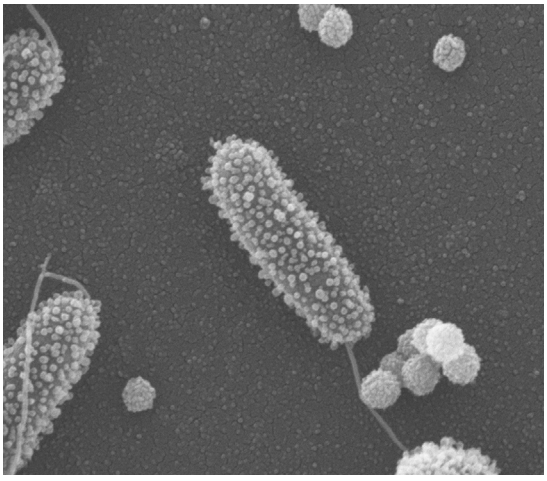

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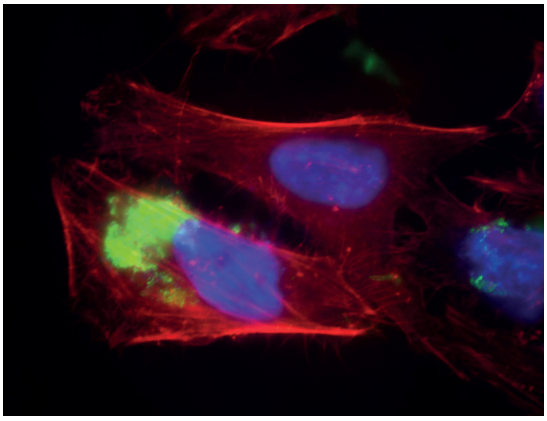

1 *Scanning electron microscopy of P. aeruginosa cells growing in conditions that stimulate type three secretion. The small spheres are extracellular vesicles.*

2 *Intracellular accumulation of ExoS toxin (green) secreted by P. aeruginosa through its type three secretion system.*

Heterologous gene expression and secretion in gram-positive bacteria with industrial applications

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The group continued to focus its research on the physiological and molecular characterisation of the main protein secretion mechanism (Sec system) of the soil Gram-positive bacteria *Streptomyces lividans,* widely used in industry as an efficient producer of extracellular hydrolytic enzymes and other compounds of industrial interest. Secretory protein overproduction triggers a secretion stress response, eliciting the synthesis of three specific proteases that degrade misfolded proteins, and a stringent response in *Streptomyces.*

Bacterial lipoproteins are a specialised class of membrane proteins reported to play a pivotal role in bacterial cell physiology, including protein folding. Absence of functional type II signal peptidase (Lsp), which cleaves the lipoprotein signal peptide, apparently produces a translocase blockage, as determined by the amount of the overproduced alpha-amylase that clearly accumulates on the internal surface of the membrane. As a result of the translocase blockage, Lsp deficiency reduces synthesis of secretory proteins in *S. lividans*, as is the case when the cell is deficient in the translocase complex (SecG mutant strain) or the major type I signal peptidase (SipY mutant strain). The *lsp* mutation also triggers a stringent response, as the absence of functional SBP lipoproteins causes non-sensing of solutes (nutrients) in the culture medium. These findings are of particular relevance with respect to characterising potential bottlenecks in *S. lividans* secretion and optimising *S. lividans* for the overproduction of secretory proteins of industrial application.

For a number of years, we have monitored the rhizobacterial communities of transgenic maize tolerant to glyphosate. In a new research line, we identified the most attractive method-combination workflow to analyse next generation sequencing results from rhizobacterial community experimental data, depending on sequence variability number and length. The worldwide increase in glyphosate-resistant weed populations led to new cultivation strategies based on combinations of pre- and post-emergence herbicides. Over a one-year cultivation cycle, we evaluated the impact of several herbicide combinations on the rhizobacterial community of glyphosate-tolerant Bt-maize and compared them to those of untreated or glyphosate-treated soils, and analysed the resilience of the microbial communities by comparing their relative composition at the end of the cultivation cycle.

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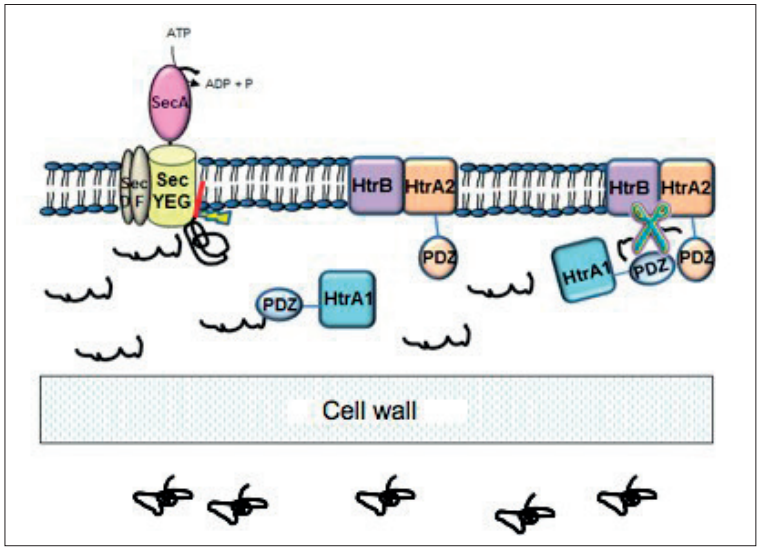

1 *Proposed model of protease action when secretory proteins are overproduced. Soluble HtrA1 recognises the misfolded proteins on the outer cytoplasmic membrane, while HtrB and HtrA2 remain in the membrane, forming heterodimers. The HtrA1-protein complex interacts with the HtrB-HtrA2 heterodimer via the PDZ domains, creating a structure able to cleave the misfolded proteins.*

Regulation of the metabolism of hydrocarbons in bacteria

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To be competitive in the environments they colonise, bacteria must optimise metabolism to attain maximum gain from available nutrients at minimum energetic cost. Not all potential carbon sources are equally effective in this respect. Probably for this reason, when confronted with a mixture of potentially assimilable compounds at sufficient concentrations, many bacteria preferentially use one of them, leaving others aside until the preferred one is consumed. This selection implies a complex regulatory process termed catabolite repression. Unravelling the molecular mechanisms that underlie these regulatory events helps to understand how bacteria coordinate their metabolism and gene expression programs and optimise growth. It also aids in the design and optimisation of biotechnological processes and to understand how bacteria degrade compounds in nature. This is particularly true for compounds that are difficult to degrade and thus accumulate in the environment, posing pollution problems. Hydrocarbons are a clear example of this kind of non-preferred compound. Catabolite repression also has an important influence on the virulence and antibiotic resistance properties of pathogenic bacteria.

Our aim is to characterise the overall regulation networks responsible for catabolite repression, identify their components, the signals to which they respond, the molecular mechanisms by which they regulate gene expression, and determine how they modulate metabolism. The regulatory proteins involved in these networks differ among microorganisms. We use *Pseudomonas putida* as an experimental model because it has a versatile metabolism, it colonises very diverse habitats, and is widely used in biotechnology. We currently focus on two catabolite repression networks. One relies on the Crc and Hfq proteins, which ultimately inhibit translation of mRNA with the specific CA sequence motif within their translation initiation region. Two small RNAs, the levels of which vary greatly depending on growth conditions, antagonise the inhibitory effect of Hfq and Crc. The other regulatory network under study receives signals from the electron transport chain, thereby coordinating respiration with metabolic needs. Finally, we found that growth temperature influences catabolite repression. At low growth temperatures, repression is relieved at some genes, but not at others. This could be relevant for several biotechnological applications.

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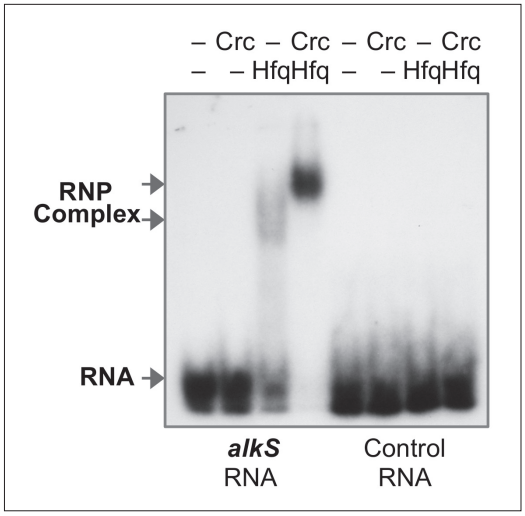

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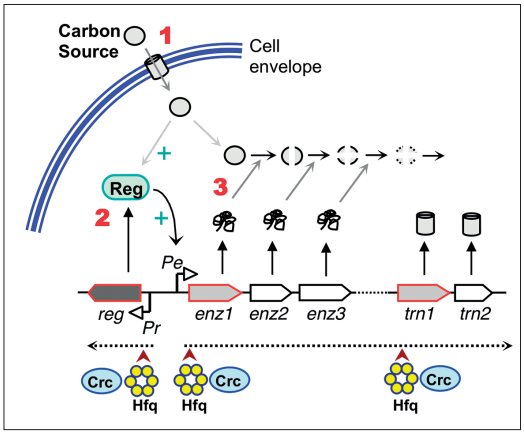

1 *Effect of Crc on Hfq protein binding to RNA oligonucleotides bearing (alkS RNA) or lacking (control RNA), a "catabolite activity" motif.*

2 *Crc and Hfq proteins can modulate the activity of catabolic pathways for carbon sources by controlling the expression of genes involved in uptake (trn) and metabolism (enz1, enz2, etc.) of the carbon source or in the regulation (reg) of catabolic genes.*

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Genetic control of the cell cycle

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Our research aims to partially reproduce in the test tube the assembly and functions of the divisome components, a dedicated set of proteins that gather at midcell and divide a bacteria into two equal daughters. Division is accurately regulated in the cell, both in time and space, ensuring that no division occurs unless all the components of the mother have been duplicated and distributed equally to each daughter. A good number of the divisome elements and a few of their functions are known, and their assembly sequence and location in the cell have been determined. This knowledge facilitates a synthetic biology approach in which a discrete number of different divisome components can be assembled and tested in artificial containers.

We use two types of containers, vesicles and maxicells. Vesicles are cell-free systems in which the desired divisome component or a mixture of them can be encapsulated and their function tested under controlled biochemical conditions. FtsZ, a tubulin analogue with a crucial role in divisome function, can be placed inside vesicles. In permeable vesicles, FtsZ can be induced to form polymers by addition of GTP, the nucleotide that supplies energy to this process in bacteria. We anchored the FtsZ protein to the inner face of the vesicles using ZipA, one of the proteins that naturally tethers it to the cytoplasmic membrane of *Escherichia coli*. Addition of GTP results in the shrinking of these vesicles.

Maxicells are *E. coli* cells in which the nucleoid is degraded by controlled irradiation with ultraviolet light. They contain no chromosome but can express those genes that have been previously introduced in plasmids that, due to their small size, escape irradiation damage. In normal cells, the nucleoid associated with the protein SlmA prevents assembly of a divisome at midcell by occlusion. Besides the nucleoid, maxicells also lack SlmA and thus do not have this occlusion mechanism. They nonetheless retain an active alternative system, a set of three Min proteins that oscillate from pole to pole, preventing FtsZ polymerisation at sites other than the centre.

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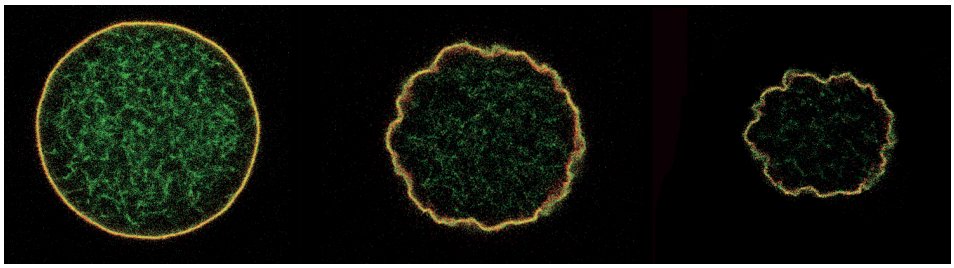

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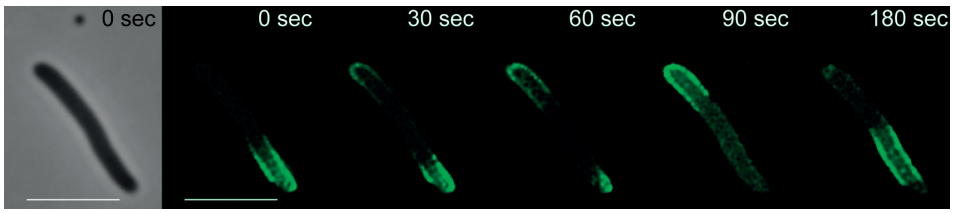

1 *Shrinkage of a vesicle with ZipA inserted into its membrane and FtsZ encased in the lumen. ZipA was labelled with a red fluorescent dye, whereas FtsZ was labelled in green. Yellow fluorescence is observed where the two proteins coincide. To initiate the experiment, GMP-CPP, a slowly hydrolysable GTP analogue, is added (left). This leads to a slow polymerisation of FtsZ, followed by progressive shrinkage of the vesicle (centre and right). Reproduced from Cabré et al. J Biol Chem 2013.*

2 *The MinD protein explores the bacterial length. A green fluorescent variant of MinD oscillates from pole to pole. MinD guides MinC, a protein able to block cell division. As both proteins travel from one pole to the other, the only site at which they are absent for a longer period is the centre. This mechanism ensures that the bacterium divides only through the middle. The image at the left shows the full image of the bacteria as seen by phase contrast microscopy. Bar = 5 μm. Modified from Pazos et al., 2014; PLoS One 9:e91984.*