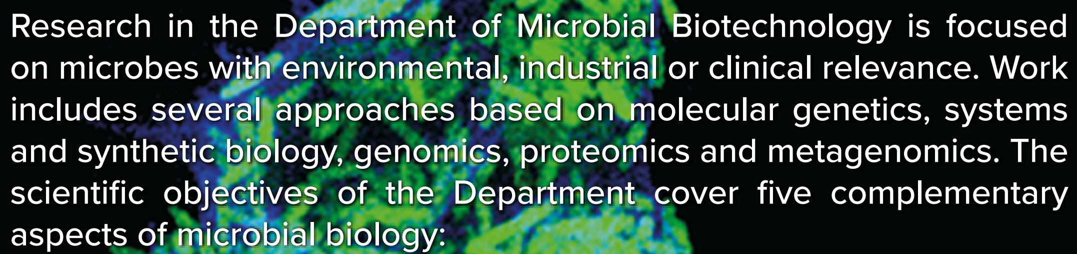

Research in the Department of Microbial Biotechnology is focused on microbes with environmental, industrial or clinical relevance. Work includes several approaches based on molecular genetics, systems and synthetic biology, genomics, proteomics and metagenomics. The scientific objectives of the Department cover five complementary aspects of microbial biology:

i. Environmental microbiology. We aim to characterise the mechanisms underlying the global regulation networks that control and coordinate bacterial metabolism, optimising the use of resources in their growth medium or environment. This will help to understand how bacteria assimilate different compounds in their environments and will allow to redesign their metabolism towards the efficient production of valuable compounds or the degradation of toxic molecules.

ii. Microbial pathogens. Efforts are directed to decipher host-pathogen interactions occurring in infections caused by a variety of microorganisms, including intracellular pathogens. Basic processes of microbial physiology, such as cell division or the formation of so called lipid-rafts, which are relevant for both infection and for defining antimicrobial targets, are studied as well.

iii. Microbial resistance to antibiotics and search for new antimicrobials. Work aims to understand the mechanisms of bacterial resistance to antibiotics and to the effect of acquiring such resistance in bacterial physiology. In addition, we search for potential targets as a way to develop new antimicrobials and study new anti-resistance molecules.

iv. Microbial responses to hostile environments. The focus is to understand how bacteria respond to stressful environments, including general stress responses and specific responses to agents causing DNA damage. We study how bacterial viruses replicate their DNA, how bacteria repair DNA damages, promote segregation to improve genome stability and horizontal gene transfer. Novel mechanisms for repairing DNA damage are currently under study.

v. Microbial engineering. The purpose is to generate bacterial strains optimised to obtain products of interest (recombinant antibodies, hydrolytic enzymes, antimicrobials). In addition, we design whole-cell synthetic bacteria that could be used for diagnostic and therapeutic applications, as well as to degrade pollutants. We engineer protein secretion systems to develop synthetic adhesins, driving the attachment of the engineered bacteria to specific target cells (e.g. tumour cells). Engineering of protein secretion nanomachines also allows for the delivery of therapeutic proteins (e.g. antibodies) in the target cell.

Microbial Biotechnology

HEAD OF DEPARTMENT

José Luis Martínez

RESEARCH GROUPS

1. **Genetic stability**
   Juan C. Alonso
2. **Recombination-dependent DNA replication**
   Silvia Ayora
3. **Stress and bacterial evolution**
   Jesús Blázquez
4. **Bacterial engineering for biomedical applications**
   Luis A. Fernández-Herrero
5. **Laboratory of intracellular bacterial pathogens**
   Francisco García-del Portillo
6. **Molecular infection biology**
   Daniel López
7. **Ecology and evolution of antibiotic resistance**
   José Luis Martínez
8. **Heterologous gene expression and secretion in gram-positive bacteria with industrial applications**
   Rafael P. Mellado
9. **Regulation of gene expression and metabolism in bacteria**
   Fernando Rojo
10. **Genetic control of cell cycle**
    Miguel Vicente

Genetic stability

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SELECTED PUBLICATIONS

Le S, Serrano E, Kawamura R, Carrasco B, Yan J, Alonso JC. *Bacillus subtilis* RecA with DprA-SsbA antagonizes RecX function during natural transformation. Nucleic Acids Res 2017; 45: 8873-8885.

Moreno-Del Álamo M, Tabone M, Lioy VS, Alonso JC. Toxin ζ triggers a survival response to cope with stress and persistence. Front Microbiol 2017; 8: 1130.

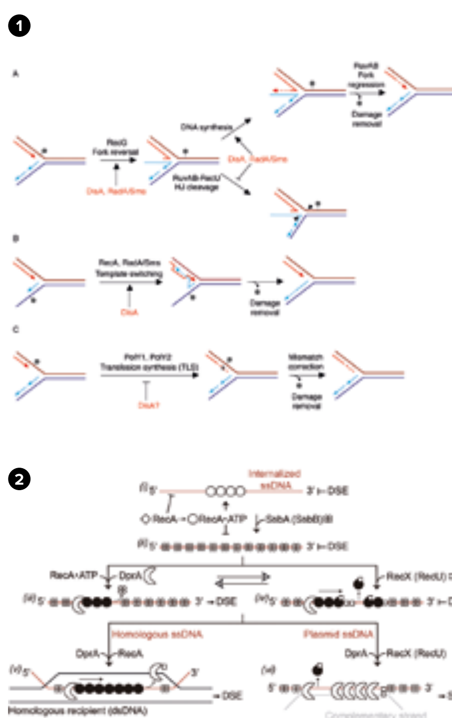
Raguse M, Torres R, Seco EM, Gándara C, Ayora S, Moeller R, Alonso JC. *Bacillus subtilis* DisA helps to circumvent replicative stress during spore revival. DNA Repair (Amst) 2017; 59: 57-68.

Serrano E, Carrasco B, Gilmore JL, Takeyasu K, Alonso JC. RecA regulation by RecU and DprA during *Bacillus subtilis* natural plasmid transformation. Front Microbiol 2018; 9: 1514.

Carrasco B, Seco EM, López-Sanz M, Alonso JC, Ayora S. *Bacillus subtilis* RarA modulates replication restart. Nucleic Acids Res 2018; 46: 7206-7220.

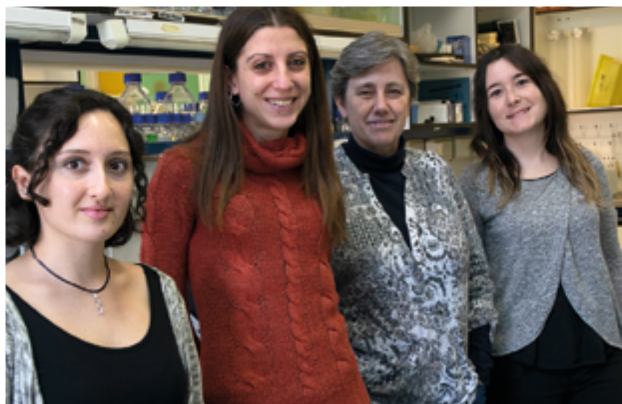
Our research focuses on the study of the molecular mechanisms that bacteria of the *Firmicutes phylum* use to secure genomic stability, to promote horizontal gene transfer, and to control cell proliferation and accurate plasmid segregation. Using *Bacillus subtilis* as a model, we have shown that the DNA damage response recruits different complex molecular machineries depending on the type of DNA damage and the growth conditions. We have shown that, in the presence of stalled or reversed forks (Holliday junction, HJ), DisA may trigger error-free and error-prone DNA damage tolerance (DDT) responses. DisA, in concert with the RecA recombinase, branch migration translocases (RecA/Sms, RecG and RuvAB) and the HJ resolvase (RecU) RadA/Sms, recognises the displaced loops and HJ intermediates and suppress c-di-AMP synthesis that in turn halts cell proliferation during exponential growth or during revival of haploid spores (Figure 1).

Starved *B. subtilis* cells develop natural competence, with DprA, SsbB, SsbA, RecO(R), RecX, and CoiA contributing to help RecA to increase genetic diversity. Studying the functions that control RecA activities, we are addressing how mediators and modulators contribute to the maintenance of the species. SsbA or SsbB inhibits RecA loading onto ssDNA. DprA enhances the polymerisation of RecA onto SsbA-coated ssDNA and RecX or RecU facilitates RecA depolymerisation from ssDNA. Activated RecA or DprA counters RecX or RecU negative regulation on RecA nucleoprotein filament assembly (Figure 2). Plasmid transformation, which is a RecA-independent event, requires RecX or RecU to promote RecA depolymerisation from the linear plasmid ssDNA, and DprA to catalyse DNA strand annealing of the complementary strands coated by SsbA or SsbB, and circularisation of the redundant tailed ends to render an active replicon.



❶ *Proposed DDT mechanisms. An unrepaired DNA lesion on the leading strand template (black dot) (A and C) or an unrepaired DNA lesion on the lagging strand (B) causes replication fork blockage. (A) DisA regulates branch migration of the stalled fork. Synthesis of the DNA complementary to the damaged site (dotted line) overcomes blockage upon fork regression. B, DisA regulates RecA-mediated strand invasion on the undamaged sister strand (template switching). C, the replicative DNA polymerase is replaced by PolY1 and/or PolY2 that catalyse nucleotide mis-incorporation (lesion bypass, denoted with X). After damage removal and mismatch correction replication continues.*

❷ *Model for RecA-mediated natural transformation. Apo RecA cannot nucleate on the ssDNA. RecA bound to ATP undergoes its first structural transition (empty circle). RecA·ATP can nucleate onto ssDNA, but it cannot catalyse DNA strand exchange (DSE) (step i). RecA·ATP cannot nucleate on the SsbA-ssDNA complexes (step ii). DprA interacts with and loads RecA onto SsbA-coated ssDNA. RecA undergoes its second transition (filled circle) E (step iii). RecU (or RecX) promotes RecA disassembly from the ssDNA (step iv). DprA assists RecA·ATP assembly and favour homologous chromosomal transformation (step v). In the absence of homology, DprA binds to the complementary plasmid strand (gray line) and catalyses single strand annealing (SSA) (step vi).*



Recombination-dependent DNA replication

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SELECTED PUBLICATIONS

Valero-Rello A, López-Sanz M, Quevedo-Olmos A, Sorokine A, Ayora S. Molecular mechanisms that contribute to horizontal transfer of plasmids by the bacteriophage SPP1. Front Microbiol 2017; 8: 1816.

Seco EM and Ayora S. *Bacillus subtilis* DNA polymerases, PolC and DnaE, are required for both leading and lagging strand synthesis in SPP1 origin-dependent DNA replication. Nucleic Acids Res 2017; 45: 830-8313.

Neamah MM, Mir-Sanchis I, López-Sanz M, Acosta S, Baquedano I, Haag AF, Marina A, Ayora S, Penades JR. Sak and Sak4 recombinases are required for bacteriophage replication in *Staphylococcus aureus.* Nucleic Acids Res 2017; 45: 6507-6519.

Khavnekar S, Dantu SC, Sedelnikova S, Ayora S, Rafferty J, Kale A. Structural insights into dynamics of RecU-HJ complex formation elucidates key role of NTR and stalk region toward formation of reactive state. Nucleic Acids Res 2017; 45: 975-986.

Carrasco B, Seco EM, López-Sanz M, Alonso JC, Ayora S. *Bacillus subtilis* RarA modulates replication restart. Nucleic Acids Res 2018; 46: 7206-72220.

Genomic instability is minimised by four major mechanisms: high-fidelity DNA replication, precise chromosome segregation, error free repair of DNA damage and coordinated cell cycle progression. Our research focuses on the study of the mechanisms that cells use to continue DNA replication when this process encounters impediments, which may eventually stall or collapse the fork, producing DNA gaps or broken DNA ends. Replication restart is then mediated by proteins that were initially identified by their roles in homologous recombination and repair of DNA double-strand breaks. We use a simple model system, *Bacillus subtilis* and its bacteriophage SPP1, and several biophysics, molecular biology and genetic techniques to study the recombination mechanisms that contribute to genome stability.

One of our lines of research focuses on the effect of recombination proteins in DNA replication. In the last years, we have reconstituted *in vitro* the replisome of *B. subtilis* and its phage SPP1. Mimicking stalled and collapsed replication forks with artificial substrates, we have analysed how the conserved RarA protein modulates replication restart. Currently, the effects of other recombination proteins in DNA replication are under study.

Recombination also leads to evolution, and recombination proteins are frequently encoded in the genome of many bacteriophages. Our study has demonstrated that viral DNA-single strand annealing proteins of the Sak, Sak4 and RecT families are absolutely required for phage DNA replication and for the horizontal transfer of plasmids, independently of their mechanism of replication. The contribution of other viral recombination proteins to DNA replication and horizontal gene transfer was also analysed.

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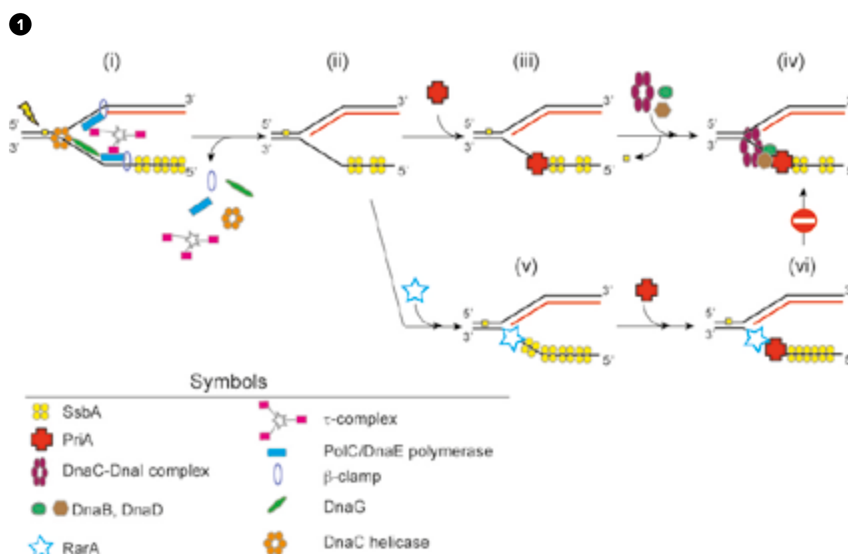

2 *Model of RarA action on blocked forks. (i) When a lesion blocks DNA replication, DNA synthesis is stopped and the replisome disassembles. (ii to iv) SsbAA-PriA load the DnaC helicase. Then the damage is repaired, and the helicase DnaC recruits DnaG primase and the replisome and DNA replication can restart . (v and vi) If the DNA damage is not repaired, SsbA loads RarA at the stalled fork. The SsbA-RarA-PriA-DNA complex impedes the recruitment of the replisome and initiation of DNA synthesis is inhibited.*

Stress and bacterial evolution

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We try to understand the genetic mechanisms involved in genome stability in bacteria and their roles in evolution and adaptation. Specifically, we study the genetic basis of both stable and induced hyper-mutation/hyper-recombination as bacterial “strategies” to speed adaptation to environmental stresses such as antibiotics.

We have discovered a novel non-canonical mismatch repair system in prokaryotes (present in Archaea and Actinobacteria), responsible for maintaining genome stability in *Mycobacterium*. Mutants in the key protein of this system, NucS, display increased mutation and recombination rates. We are trying to disentangle its genetics and biochemical bases in *Mycobacterium* and *Streptomyces*.

We are applying this knowledge to i) understand and prevent the development of antibiotic resistance in bacterial pathogens (including *Escherichia coli, Pseudomonas aeruginosa, Acinetobacter baumannii, Mycobacterium abscessus* and *Mycobacterium tuberculosis*) and ii) improve prokaryotic species of biotechnological interest (including *Streptomyces, Mycobacterium, Bifidobacterium, Rhodococcus, Corynebacterium, Pyrococcus* and *Halobacterium*).

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Castañeda-García A, Prieto AI, Rodríguez-Beltrán J, Alonso N, Cantillon D, Costas C, Pérez-Lago L, Zegeye ED, Herranz M, Plociński P, Tonjum T, García de Viedma D, Paget M, Waddell SJ, Rojas AM, Doherty AJ, Blázquez J. A non-canonical mismatch repair pathway in Prokaryotes. Nat Commun 2017; 8: 14246.

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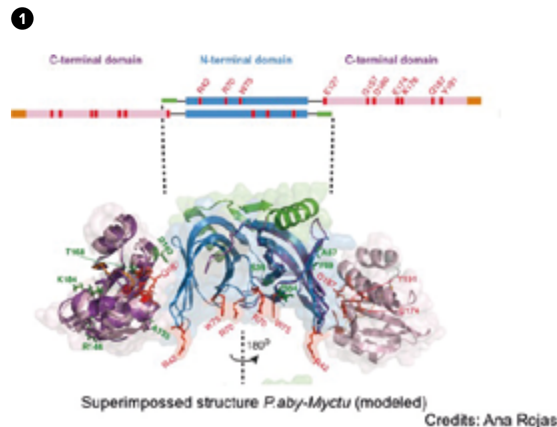

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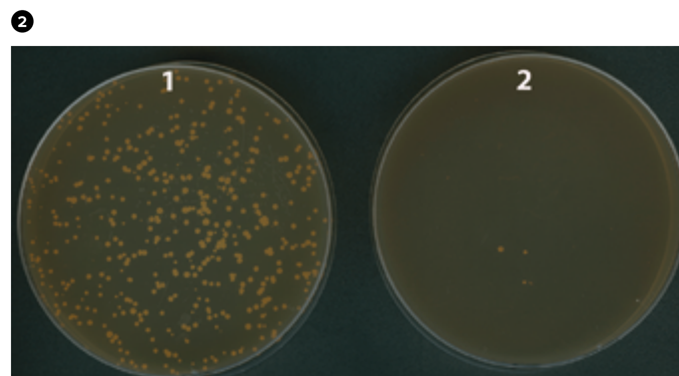

❶ *Domain characterisation of M. tuberculosis NucS, the key protein of the novel non-canonical mismatch repair system. Credits: Ana Rojas.*

❷ *NucS is a guardian of the genome stability in Mycobacterium. Increased production of mutant clones resistant to rifampicin in a* Mycobacterium smegmatis *strain lacking nucS gene (1) in comparison with the nucS-proficient strain (2).*

Bacterial engineering for biomedical applications

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Ronald Jara Gacitua

SELECTED PUBLICATIONS

Álvarez B and Fernández LA. Sustainable therapies by engineered bacteria. Microb Biotechnol 2017; 10: 1057-1061.

Salema V and Fernández LA. *Escherichia coli* surface display for the selection of nanobodies. Microb Biotechnol 2017; 10: 1468-1484.

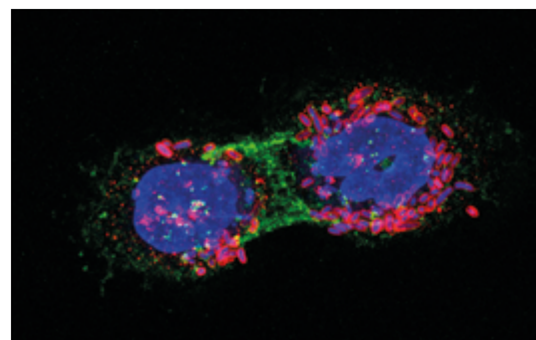
Cepeda-Molero M, Berger CN, Walsham A, Ellis S, Wemys-Holden S, Schüller S, Frankel G, Fernández LA. Attaching and effacing (A/E) lesion formation by enteropathogenic *E. coli* on human intestinal mucosa is dependent on non-LEE effectors. PLoS Pathog 2017; 13: e1006706.

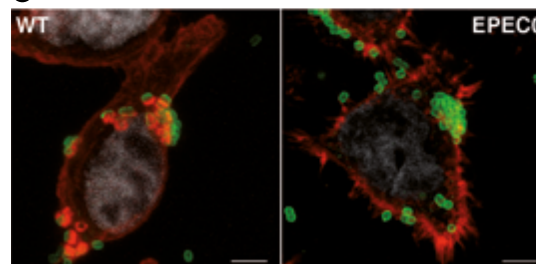
Kalograiaki I, Abellán Flos M, Fernández LA, Menéndez M, Vincent S, Solís D. Direct evaluation of live uropathogenic *E. coli* adhesion and efficiency of anti-adhesive compounds using a simple microarray approach. Anal Chem 2018; 90: 12314-12321.

Our research is aimed to engineer *E. coli* bacteria for biomedical applications, including the selection of small recombinant antibodies and the design of bacteria for diagnostic and therapeutic use *in vivo*. We study protein secretion systems found in pathogenic *E. coli* strains, such as enteropathogenic *E. coli* (EPEC), and engineer them to develop protein nanomachines that can be applied for selection of recombinant antibodies and the delivery of therapeutic proteins by non-pathogenic *E. coli* strains. Among the recombinant antibodies, we employ single-domain antibodies (sdAbs) or nanobodies, the smallest antibody fragments known-to-date with full antigen-binding capacity. We use synthetic biology approaches and genome engineering to combine the expression of these modular parts in the designed bacteria.

The specific projects in which we have worked are:

1. ***E. coli* display technology for selection of nanobodies from libraries.** Bacterial outer membrane proteins of the Intimin-Invasin and autotransporter families have been used to display nanobody libraries on the surface of *E. coli* and for the selection of high-affinity binders against antigens relevant in infection diseases and cancer.
2. **Re-programming *E. coli* adhesion to tumours with synthetic adhesins.** The display of nanobodies on the surface of *E. coli* has allowed us to generate "synthetic adhesins" that can drive the attachment of bacteria to target tumour cells expressing cell surface antigens.
3. **Injection of therapeutic proteins from *E. coli* into human cells.** We are engineering the type III protein secretion system (T3SS) from EPEC to directly deliver therapeutic proteins and nanobodies from *E. coli* into the cytosol of tumour cells. We are engineering the controlled expression of EPEC T3SS in the non-pathogenic *E. coli* K-12 strain to specifically deliver cytotoxins in tumour cells. In addition, we have generated an effector-less EPEC strain (EPEC0) that can inject specific combination of proteins of interest to human cells.





❶ *Fluorescence confocal microscopy image showing* E. coli *bacteria (red fluorescence) with synthetic adhesins targeting an antigen (green fluorescence) expressed on the surface of human tumour cells (nuclei and bacterial DNA stained in blue).*

❷ *Fluorescence confocal microscopy image showing enteropathogenic* E. coli *(EPEC) (green fluorescence) wild type (WT) and effector-less strain (EPEC0) infecting HeLa cells. EPEC0 bacteria do not inject any effector present in the pathogen, like the translocated intimin receptor Tir that polymerises F-actin under the attached bacteria. (F-actin in red; nuclei in grey).*

Laboratory of intracellular bacterial pathogens

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María Laura Echarren (Argentine)
Isela Serrano (Mexico)

SELECTED PUBLICATIONS

Castanheira S, *et al*. A specialized peptidoglycan synthase promotes Salmonella cell division inside host cells. MBio 2017; 8: e01685-17.

Impens F, *et al*. N-terminomics identifies Prli42 as a membrane miniprotein conserved in Firmicutes and critical for stressosome activation in Listeria monocytogenes. Nat Microbiol 2017; 2: 17005.

García-del Portillo F and Pucciarelli MG. RNA-Seq unveils new attributes of the heterogeneous Salmonella-host cell communication. RNA Biol 2017; 14: 429-435.

Castanheira S, Cestero JJ, García-del Portillo F, Pucciarelli MG. Two distinct penicillin binding proteins promote cell division in different Salmonella lifestyles. Microb Cell 2018; 5: 165-168.

Casino P, Miguel-Romero L, Huesa J, García P, García-del Portillo F, Marin A. Conformational dynamism for DNA interaction in the Salmonella RcsB response regulator. Nucleic Acids Res 2018; 46: 456-472.

Our lab aims to understand the physiological changes that accompany the adaptation to an intracellular lifestyle of the model pathogens *Listeria monocytogenes* and *Salmonella enterica*, both causing food-borne diseases of high incidence in humans and livestock.

During these two years, we have focused on deciphering mechanisms responsible for: i) the adaptation of *Listeria monocytogenes* to stress conditions such as high osmolarity and cold (4ºC), as well as the assembly dynamics of the 'stressosome' complex; and, ii) the remodelling of the main cell wall component, peptidoglycan, in *Salmonella enterica* when this pathogen inhabits the intracellular niche of eukaryotic cells. These studies have allowed us to unravel novel phenomena with no precedents in the literature.

These novel findings include the induction of a subset of cell surface proteins and regulatory small RNAs (sRNAs) when *L. monocytog*enes grows at 4ºC and the chemical modification of the peptidoglycan when *S. enterica* persists inside vacuolar compartments of eukaryotic cells. We have also obtained preliminary evidence linking these peptidoglycan modifications to alteration of defence responses in the infected cell.

Most of our current interest is now directed to: i) unravel the global regulatory network of *L. monocytog*enes at 4ºC (temperature at which this food-borne pathogen proliferates) using RNASeq approaches and classical genetics; ii) assess how the stressosome complex of *L. monocytog*enes responds to the intracellular infection; iii) identify the enzymatic activities of *S. enterica* that alter peptidoglycan structure in response to host signals; and, iv) define how intra-vacuolar *S. enterica* interferes host innate immunity signalling to establish a persistent infection.

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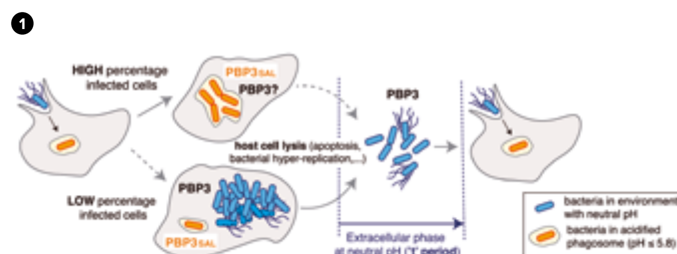

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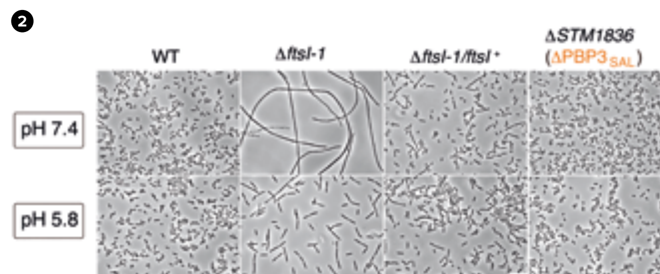

❶ *Model proposed for the difference use of penicillin-binding proteins PBP3 and PBP3SAL by* Salmonella enterica *during the course of the infection. Note that PBP3SAL promotes cell division of the pathogen inside acidic phagosomes of eukaryotic cells.*

❷ *Phenotype of a* Salmonella enterica *mutant lacking the ftsI gene that encodes penicillin binding 3 (PBP3), a peptidoglycan enzyme essential for cell division in* Escherichia coli. *Note that this ΔftsI mutant, which can only use the Salmonella-specific PBP3SAL for building the division septum, divides exclusively in acidified media.*

Molecular infection biology

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SELECTED PUBLICATIONS

García-Fernandez E, Koch G, Wagner RM, ... López D. Membrane microdomain disassembly inhibits MRSA antibiotic resistance. Cell 2017; 171: 1354-1367.

Mielich-Süß B, ... López D. Flotillin scaffold activity contributes to type VII secretion system assembly in *Staphylococcus aureus.* PLoS Pathog 2017; 13: e1006728.

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Koch G, Wermser Ch, Acosta IC, Kricks L, ... López D. Attenuating *Staphylococcus aureus* virulence by targetting flotillin protein scaffold activity. Cell Chem Biol 2017; 24: 845-857.

Dragos A, Martin M, Falcón-Garcia C, Kricks L, ... López D, Lieleg O & Kovács AT. Collapse of genetic division of labour and evolution of autonomy in pellicle biofilms. Nat Microbiol 2018; 3: 1451–1460.

A number of bacterial cell processes are confined in platforms termed functional membrane microdomains, some of whose organisational and functional features resemble those of lipid rafts of eukaryotic cells. How bacteria organise these intricate platforms and their biological significance remains an important question. Our laboratory is a key laboratory in the field of functional membrane microdomain bacterial compartmentalisation and its role during infections, using MRSA (Methicillin-resistance *Staphylococcus aureus*) as model organism. Our research is supported by competitive funding, such as ERC-StG-2013 or H2020 RIA Biotech-03-2016. We aim to identify the structure and molecular mechanisms that lead to bacterial membrane compartmentalisation and their role during staphylococcal infections that are resistant to antibiotic treatments. To this end, we work at the interface of molecular and cellular biology with other scientific disciplines, such as structural, infection, synthetic and systems biology. This interactive and multidisciplinary environment provides to my laboratory a means to open novel areas of research, unravel the mechanisms of bacterial infections and discover innovative antimicrobial strategies to fight antibiotic resistance and multi-drug resistance pathogens, with special emphasis on those associated with hospital infections.

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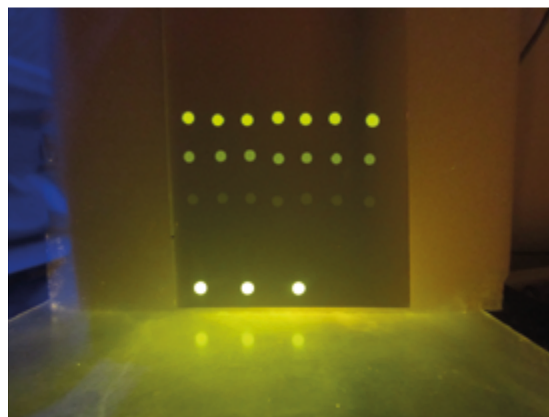

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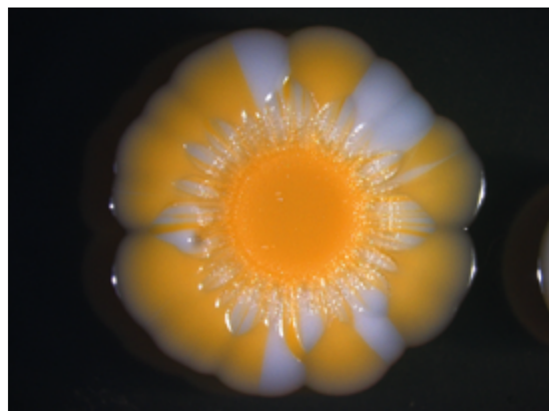

1 *Several different fluorescently labeled lipids spotted on a TLC plate and visualised under blue light.*

2 *Competitive behavior of two distinct strains of* Staphylococcus aureus *growing in a single macrocolony.*

Ecology and evolution of antibiotic resistance

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SELECTED PUBLICATIONS

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We study the biology of opportunistic pathogens, focusing on the networks and the evolutionary processes that connect resistance and virulence. In particular, in the last years we have proposed several rules for predicting the emergence of antibiotic resistance and are currently standardising these tools, which are based on experimental evolution, whole-genome sequencing and functional assays. One important element in our studies is determining the stochastic and deterministic elements that modulate the evolutionary trajectories towards antibiotic resistance of bacterial pathogens. Using this approach, we characterised mechanisms of resistance to latest-generation antibiotics and identified the target and the mechanisms of resistance to antibiotics still under development.

Since evolution towards resistance presents some degree of predictability, it is important to address the elements shifting stochastic evolution towards determinism. Among them, we are particularly interested in the epistatic interactions between antibiotics resistance elements and also between elements involved in antibiotic resistance and virulence of bacterial pathogens. Finally, we are currently studying the epigenetic events leading to transient resistance, in particular the signals that trigger such resistance, as well as identification of the differential elements modulating antibiotic resistance at the populational and the single cell levels.

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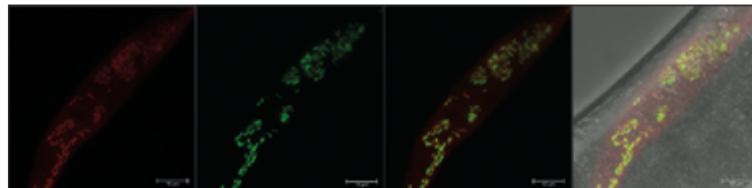

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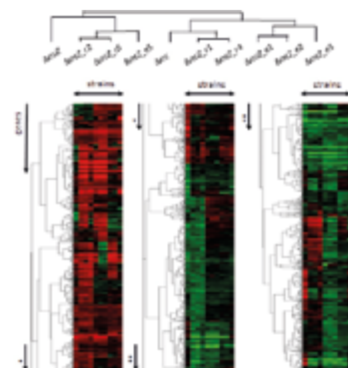
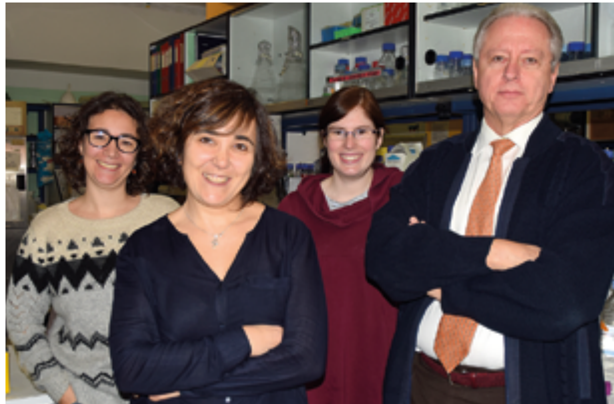


❶ *Heterogeneous gene expression of* Pseudomonas aeruginosa *cells colonising the gut of* Caenorhabditis elegans.

❷ *Transcriptional analysis of a crcZ-deficient* Pseudomonas *mutant in comparison with pseudo-revertant mutants selected in complex growth medium.*



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

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Gullón S and Mellado RP. The cellular mechanisms that ensure an efficient secretion in Streptomyces. Antibiotics (Basel) 2018, 7: E33.

Valverde JR, Gullón S, Mellado RP. Modelling the metabolism of protein secretion through the Tat route in *Streptomyces lividans*. BMC Microbiol 2018; 18: 59.

Our group has a long-standing interest in the physiological and molecular characterisation of the protein secretory routes of the soil Gram-positive bacteria *Streptomyces lividans*, a well-known efficient producer of extracellular hydrolytic enzymes and other compounds of industrial application.

We are currently advancing in the characterisation of the components affecting the quality and quantity of the protein secreted in *S. lividans*. Hence, we are studying the functioning of the three specific proteases acting in a cooperative manner (Vicente, Gullón, Marín and Mellado, PLoS One 2016; 11: e0168112), which degrade the secreted misfolded or unfolded polypeptides extracellularly accumulating outside the cell when overproduced, presumably, forming part of the quality control factors present in the bacteria. Moreover, we are characterising the bacterial proteins involved in the correct folding of the secreted proteins (peptidyl-prolyl cis-trans isomerases and thiol-disulphide oxidoreductases) to warrant a suitable balance between all of them so as to ensure an efficient secretion of the oversynthesised secretory proteins.

We also look at the structural characteristics that may favour the secretion of proteins via the major secretory route (Sec), which releases incorrectly folded proteins versus the minor route (Tat) which releases properly folded polypeptides to the culture medium, thereby facilitating the engineering of the most potentially effective transport routes to the culture medium.

Additionally, we are using metabolic flux analyses to study the metabolic changes induced by secretory protein overproduction. This will allow us to estimate the metabolic cost of that overproduction which, in turn, would enable us to design secretory protein production processes, consisting of a suitable balance between the amount of product obtained and its relative cost.

The obtained results would be applied first-hand at an industrial level for scaling up secretory protein production, allowing its optimisation, as well as favouring the design and construction of new and efficient secretory strains in *S. lividans*.

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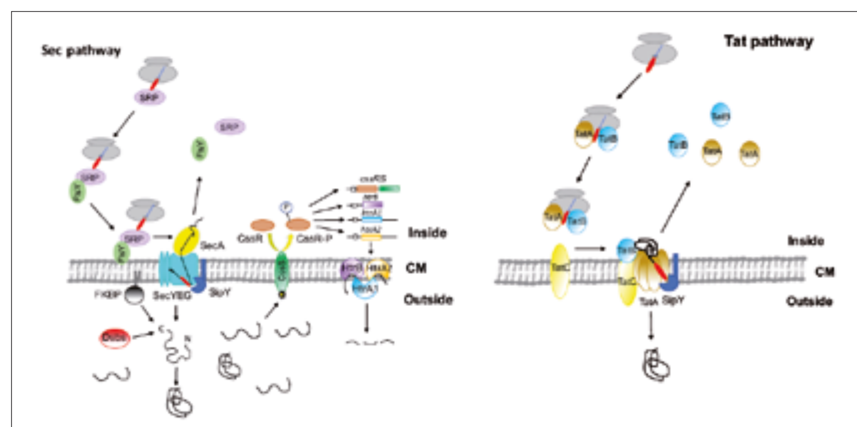
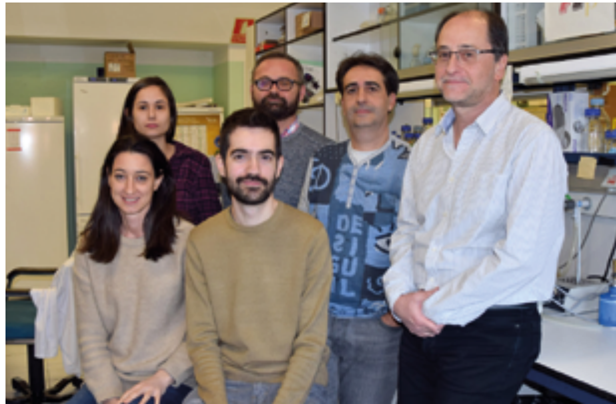


1 Streptomyces lividans *major secretory route (Sec pathway, left) releases secretory proteins out of the cell in an unfolded configuration; the action of thiol-disulphide oxidoreductases (Dsbs) and peptidyl-prolyl cis-trans isomerases (FKBP) procure the correctly folded structure that will protect secretory proteins from degradation by three specific HtrA-like proteases. The minor secretory route (Tat pathway, right) releases secretory proteins properly folded.*



Regulation of gene expression and metabolism in bacteria

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SELECTED PUBLICATIONS

Quiroz-Rocha E, Moreno R, Hernández-Ortíz A, Fragoso-Jiménez JC, Guzmán J, Rojo F, Espín G, Núñez C. Glucose uptake in *Azotobacter vinelandii* occurs through a GluP transporter that is under the control of the CbrA/CbrB-Crc/Hfq system. Sci Rep 2017; 7: 858.

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Val-Calvo J, Luque-Ortega JR, Crespo I, Miguel-Arribas A, Abia D, Sánchez-Hevia D, Serrano E, Gago-Córdoba C, Ares S, Alfonso C, Rojo F, Wu LJ, Boer R, Meijer WJJ. Novel regulatory mechanism of establishment genes of conjugative plasmids. Nucleic Acids Res 2018; 46: 11910-11926.

To be competitive in the environments they colonise, bacteria must optimise their 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 accumulate in the environment, posing pollution problems. Hydrocarbons are a clear example of this kind of non-preferred compounds.

The regulatory proteins and molecular mechanisms responsible for catabolite repression differ among microorganisms. Our work is focused on *Pseudomonas putida*, a bacterium that has a very versatile and robust metabolism, colonises very diverse habitats, and is widely used in biotechnology. In the last few years we have been analysing a regulatory network that relies on the Crc and Hfq proteins, which ultimately inhibit translation of mRNAs containing a specific A-rich sequence motif within their translation initiation region. Two small RNAs named CrcZ and CrcY, the levels of which vary greatly depending on growth conditions, antagonise the inhibitory effect of Hfq and Crc. Our aim is to characterise the influence of Crc, Hfq, CrcZ and CrcY in the physiology of *P. putida*, the signals to which they respond, and the molecular mechanisms by which they regulate gene expression. We pretend to determine how they modulate metabolism in response to fluctuating environmental conditions.

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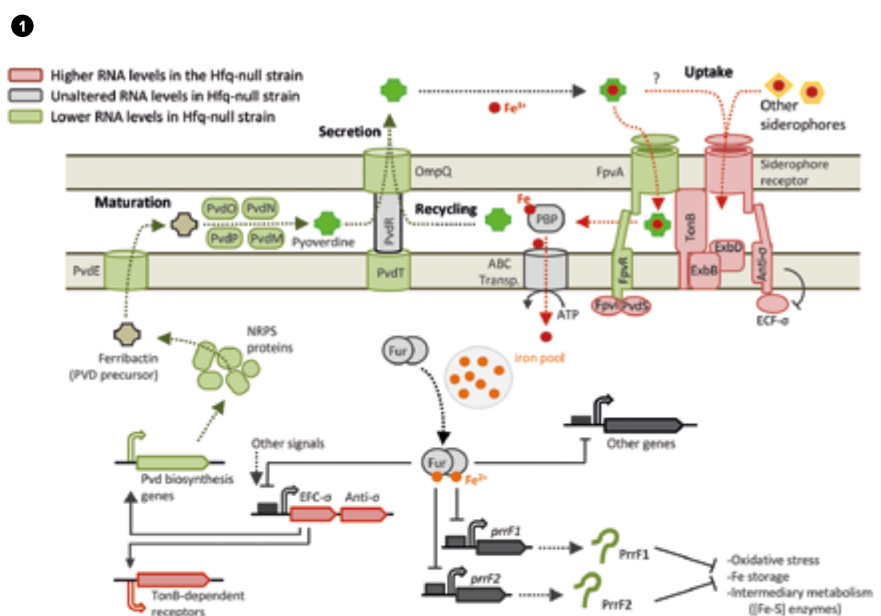

1 *Effect of the Hfq global regulator on the expression of genes involved in iron metabolism in* Pseudomonas putida. *Scheme of the synthesis, export and uptake of pyoverdine, and involvement of the Fur protein and the PrrF1 and PrrF2 sRNAs in the regulation of iron homeostasis.*

Genetic control of the cell cycle

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SELECTED PUBLICATIONS

Mura A, Fadda D, Perez AJ, Danforth ML, Musu D, Rico AI, Krupka M, Denapaite D, Tsui HT, Winkler ME, Branny P, Vicente M, Margolin W, Massidda O. Roles of the essential protein FtsA in cell growth and division in *Streptococcus pneumoniae*. J Bacteriol 2017; 199: e00608-16.

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The success of bacteria results from their ability to survive and multiply even under adverse conditions. To proliferate, *Escherichia coli*, an important inhabitant of the human gut, assembles a very efficient complex, called divisome. The divisome is exactly placed at the centre of the cell, avoiding the region occupied by the chromosome until it is fully replicated and segregated. A main component of the divisome is FtsZ, an analogue of the human tubulin, that polymerises forming a contractile ring to initiate cell division. FtsZ needs two proteins, ZipA and FtsA, to be anchored to the membrane. In addition, FtsZ is prevented by other proteins called Min from polymerising at the poles, whereas another protein SlmA, excludes the polymers from the region around the chromosome. On the other hand, a group of Zap proteins, as ZapC, serve to stabilise the FtsZ polymers. All these proteins that interact with FtsZ have collectively received the name of "The Keepers of the Ring".

Differently from their role in the division of *E. coli*, in *Streptococcus pneumoniae*, a bacterium having a coccal rather than bacillar shape, FtsA and FtsZ coordinate both peripheral and septal PG synthesis and are codependent for their localisation at midcell.

Although *E. coli* cells can grow in the absence of FtsZ, they cannot divide and form filaments. In addition, the very low levels of FtsZ present in synthetically FtsZ-deprived cells have unexpected and severe pleiotropic effects on the global physiology of *E. coli*, culminating in a reduced resilience that compromises bacterial survival. Studying the properties of FtsZ and its keepers, offers then the bright possibility to discover compounds to neutralise the ring. These would be the much needed new antibiotics essential to fight against the Dark Powers of the Ring, the antibiotic resistant pathogens.

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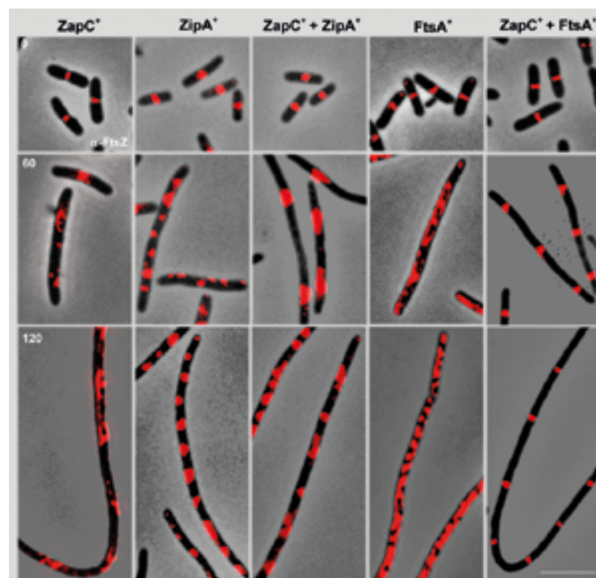

1 *The assembly of FtsZ to produce defined division rings is modified by an excess of ZapC, FtsA or ZipA. Only the combination of ZapC with FtsA (right column) allows the production of otherwise inactive rings. The frames show images of* E. coli *cells in which the FtsZ protein (red) is visualised. Each row corresponds to different times (indicated in minutes) after the induction of the corresponding genes cloned in suitable plasmids. The FtsZ protein was revealed using anti-FtsZ and Alexa 594-conjugated anti-rabbit antibody. Bar: 5 µm. (From Ortiz et al., 2017).*