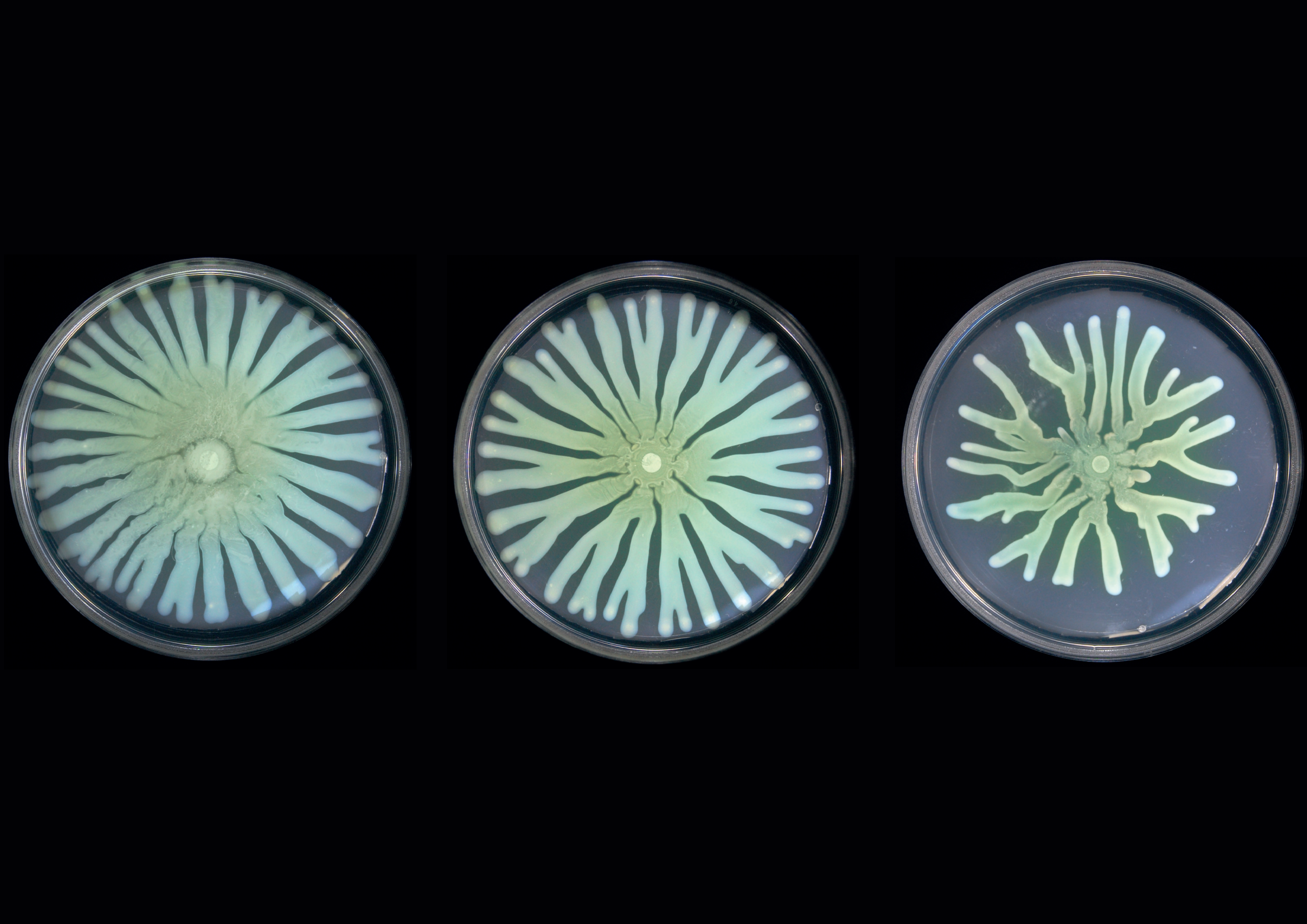


3

MICROBIAL BIOTECHNOLOGY

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 centre on five complementary aspects of microbial biology:

• Environmental microbiology. We aim to understand the regulatory mechanisms that govern the degradation of organic pollutants by analysing global regulation networks, which control the hierarchical assimilation of nutrients in complex environments. Understanding the overall regulation of bacterial metabolism will allow optimization of bioremediation strategies and industrially important biotransformation processes. Metagenomic approaches are used to evaluate the effect of natural ecosystems on the origin, enrichment and spread of antibiotic resistance, as well as on the role of waste treatment plants in alleviating this problem.

• Microbial pathogens. Efforts are directed to the host-pathogen interactions in infections caused by different types of pathogens. Basic processes of microbial physiology, such as cell division, which are relevant for infection and for defining antimicrobial targets, are studied as well.

• Microbial resistance to antibiotics and search for new antimicrobials. Work aims to define the mechanisms of bacterial resistance to antibiotics and the effect of acquiring such resistance on bacterial physiology (including virulence). In addition, we search for potential targets as a way to develop new antimicrobials.

• Microbial responses to hostile environments. The focus is on understanding 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. Novel DNA damage repair systems are currently being studied.

• Microbial engineering. The purpose is to generate bacterial strains optimized to obtain products of interest (recombinant antibodies, hydrolytic enzymes), or to detect and degrade pollutants. In addition, we engineer bacterial strains that attach to specific surfaces, including human cells expressing a surface antigen, a frequent situation in tumour cells that express proteins abnormally on the plasma membrane.

HEAD OF DEPARTMENT

José Luis Martínez

OUR RESEARCH GROUPS

1. **Genetic stability**
   Juan C. Alonso
2. **Recombination-dependent DNA replication**
   Silvia Ayora
3. **Stress and bacterial evolution**
   Jesús Blázquez
4. **Cell cycle, DNA replication, and genome instability in eukaryotes**
   Arturo Calzada
5. **Bacterial engineering for biomedical applications**
   Luis Á. Fernández
6. **Intracellular bacterial pathogens**
   Francisco García del Portillo
7. **Molecular infection biology**
   Daniel López
8. **Opportunistic pathogens**
   José Luis Martínez
9. **Heterologous gene expression and secretion in Gram-positive bacteria with industrial applications**
   Rafael P. Mellado
10. **Regulation of the metabolism of hydrocarbons in bacteria**
    Fernando Rojo
11. **Genetic control of cell cycle**
    Miguel Vicente

The level of expression of the multidrug efflux pump MexEF-OprJ modulates the motility of *P. aeruginosa*. From to to bottom, strain that does not produce the efflux pump, wild-type strain, strain that overexpesses the efflux pump. Image courtesy of Manuel Alcalde.

Genetic stability

GROUP LEADER

Juan C. Alonso

SENIOR SCIENTIST

Begoña Carrasco

POSTDOCTORAL SCIENTISTS

María Moreno del Álamo
Andrea Volante

PHD STUDENTS

Carolina Gandara
Hector Romero
Ester Serrano
Mariangela Tabone
Rubén Torres

TECHNICIAN

Chiara Marchisone

UNDERGRADUATE STUDENTS

Agnieszka Gadecka
Kaja Karas
Constanza Fuchs Gallego

SELECTED PUBLICATIONS

Volante A, Alonso JC. Molecular anatomy of ParA-ParA and ParA-ParB interactions during plasmid partitioning. J Biol Chem 2015; 290: 18782-95

Carrasco B, Yadav T, Serrano E, Alonso JC. *Bacillus subtilis* RecO and SsbA are crucial for RecA-mediated recombinational DNA repair. Nucleic Acids Res 2015; 43: 5984-5997

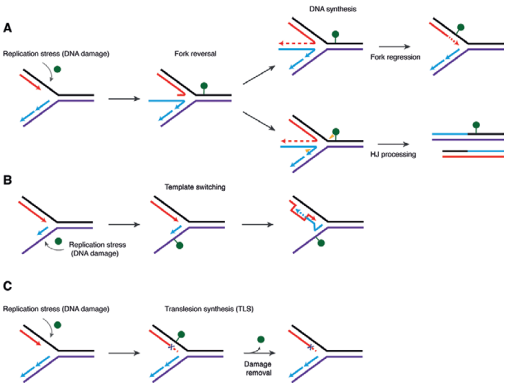
Volante A, Carrasco B, Tabone M, Alonso JC. The interaction of omega with the RNA polymerase delta' subunit functions as an activation to repression switch. Nucleic Acids Res 2015; 43: 9249-9261

Carrasco B, Serrano E, Sánchez H, Wyman C, Alonso JC. Chromosomal transformation in *Bacillus subtilis* is a non-polar recombination reaction. Nucleic Acids Res 2016 44: 2754-2768

Carrasco B, Escobedo S, Alonso JC, Suárez JE Modulation of *Lactobacillus casei* bacteriophage A2 lytic/lysogenic cycles by binding of Gp25 to the early lytic mRNA. Mol Microbiol 2016; 99: 328-337

Our research focuses on study of the molecular mechanisms that bacteria of the phylum Firmicutes use to secure genomic stability, promote horizontal gene transfer, and 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 DNA damage type. We previously found that RecN, PNPase, AddAB or the RecJ-RecQ(RecS)-SsbA complexes, the RecA-mediators (SsbA, RecOR) and modulators (RecF, RecX, RecU, RecD) help the RecA recombinase to form a nucleoprotein filament and induce the SOS response at double-strand breaks. Now we show that, in the presence of stalled or reversed forks (Holliday junction, HJ), DisA –in concert with RadA/Sms— recognizes the displaced loops and HJ intermediates and suppresses c-di-AMP synthesis, which in turn halts cell proliferation. DisA might trigger error-free and error-prone DNA damage tolerance responses in the presence of reversed fork (HJ) structures (Fig. 1). Starved *B. subtilis* cells develop natural competence, with DprA, SsbB, SsbA, RecO(R), RecX, and CoiA helping RecA to increase genetic diversity. In our study of the functions that control RecA activities, we address how recombination functions contribute to the maintenance of the species. SsbA or SsbB inhibits RecA loading onto ssDNA, and DprA, in concert with SsbA, recruits RecA and activates its DNA strand exchange activity (Fig 2). DprA enhances polymerization of RecA on SsbA-coated ssDNA, and RecX facilitates RecA depolymerization of ssDNA. Both DprA and SsbA (positively) and RecX (negatively) regulate RecA-mediated chromosomal transformation (Fig 2). For plasmid transformation, which is a RecA-independent event, RecX must promote RecA depolymerization from the linear plasmid ssDNA, and DprA must catalyse DNA strand annealing of the SsbA- or SsbB-coated complementary strands and circularization of the redundant tailed ends to render an active replicon.

1



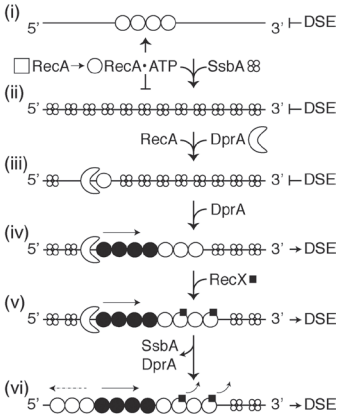
1

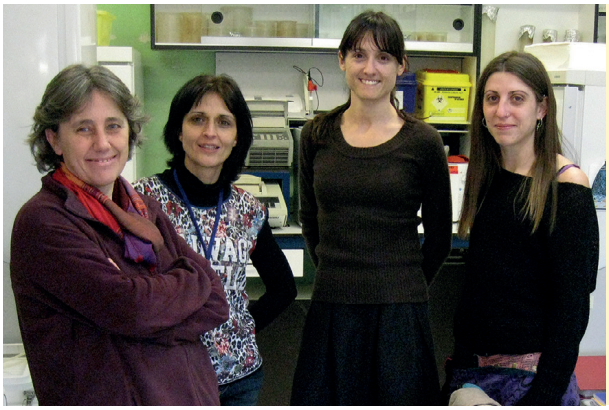
Schematic Model of DisA-mediated DNA damage tolerance. A DNA lesion (green dot) causes blockage of replication fork movement. (A) DisA coordinates RecG-mediated reversal of the stalled fork, and after DNA synthesis (dotted line), and RuvAB-mediated fork regression, DisA regulates RecU-mediated HJ cleavage. (B) The nascent strand invades the opposite newly synthesized sister strand (template switching). (C) A low-fidelity TLS DNA polymerase allows replication to proceed (lesion bypass).

2

Model for RecA filament assembly. RecA (empty square) binds ATP (oval) and ssDNA. DprA partially dislodges SsbA and loads RecA onto ssDNA. DprA and SsbA make RecA active to catalyse strand exchange (DSE). RecX interacts with RecA and dislodges it from ssDNA. DprA and SsbA proteins provide a positive contribution to RecA assembly enhancing the proficiency of RecA.

2





Recombination-dependent DNA replication

GROUP LEADER
Silvia Ayora

SENIOR SCIENTIST
Elena M. Seco

PHD STUDENT
Verónica Alonso

MASTER'S STUDENT
Sonia Acosta

TECHNICIAN
María López Sanz

UNDERGRADUATE STUDENT
Sara Cristóbal

VISITING SCIENTIST
Ana Valero
(INRA, France)

Genomic instability, a major driving force for tumourigenesis, is minimized 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 mechanisms cells use to continue DNA replication when this process encounters impediments, which might eventually stall or collapse the fork, producing DNA gaps or broken DNA ends. Replication restart is then mediated by proteins that were initially identified for their roles in homologous recombination and DNA double-strand break repair. 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 lead to replication restart.

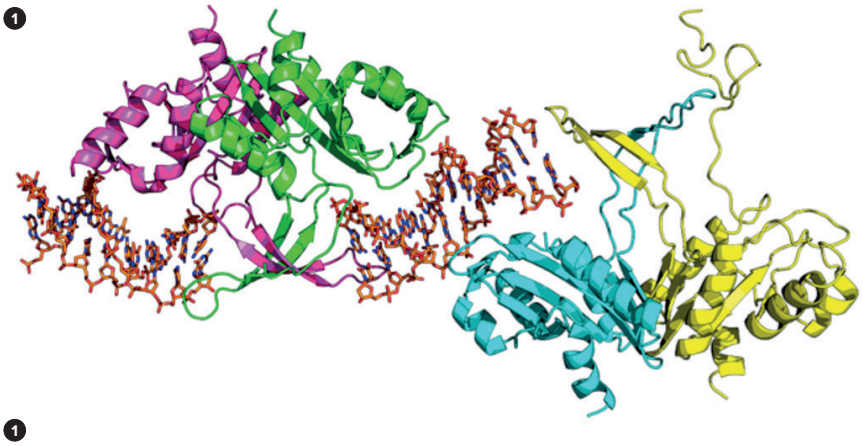
One of our research lines focuses on biochemical study of the resolution of the four-strand recombination intermediate known as the Holliday junction. In collaboration with crystallographers, we recently resolved the structure of the RecU Holliday junction-resolving enzyme bound to duplex DNA (Figure 1).

The second line of research centres on the effect of recombination proteins in DNA replication. We reconstituted *in vitro* the replisome of *B. subtilis* bacteriophage SPP1 sigma- and theta-type replication. We analysed the negative effect of RecA, the central player in homologous recombination in DNA replication. We are currently studying the effect of other recombination proteins in DNA replication.

SELECTED PUBLICATIONS

Condezo GN, Marabini R, Ayora S, Carazo JM, Alba R, Chillón M, San Martín C. Structures of Adenovirus Incomplete Particles Clarify Capsid Architecture and Show Maturation Changes of Packaging Protein L1 52/55k. J Virol 2015; 89: 9653-9664

Lioy VS, Volante A, Soberón NE, Lurz R, Ayora S, Alonso JC. ParAB Partition Dynamics in Firmicutes: Nucleoid Bound ParA Captures and Tethers ParB-Plasmid Complexes. PLoS One 2015; 10: e0131943



1

1 The crystallographic asymmetric unit (ASU) in the crystal structure of the RecU-DNA complex consists of four RecU monomers that form two dimers bound to two DNA duplexes.

Stress and bacterial evolution

GROUP LEADER

Jesús Blázquez

POSTDOCTORAL SCIENTIST

Alfredo Castañeda García

VISITING SCIENTIST

Leticia Busato Migliorini

TECHNICIAN

Samuel García Poveda

SELECTED PUBLICATIONS

Couce A, Rodríguez-Rojas A, Blazquez J. Bypass of Genetic Constraints During Mutator Evolution to Antibiotic Resistance. Proc Biol Sci 2015 282: 20142698

Rodríguez-Beltrán J, Tourret J, Tenaillon O, López E, Bourdelier E, Costas C, Matic I, Denamur E, Blázquez J. High recombinant frequency in extra-intestinal pathogenic *Escherichia coli* strains. Mol Biol Evol 2015; 32: 1708-16

Rodríguez-Beltrán J, Cabot G, Valencia EY, Costas C, Bou G, Oliver A, Blázquez J. N-acetylcysteine selectively antagonizes the activity of imipenem in *Pseudomonas aeruginosa* by an OprD-mediated mechanism. Antimicrob Agents Chemother 2015; 59: 3246-3251

Couce A, Rodríguez-Rojas A, Blazquez J. Determinants of genetic diversity of spontaneous drug-resistance in bacteria. Genetics 2016; 203: 1369-80

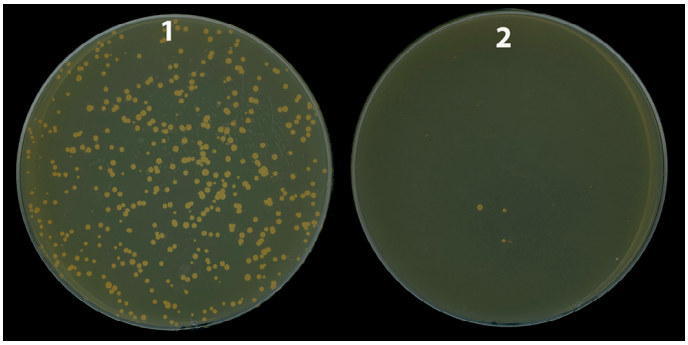
Martín-Gutiérrez G, Rodríguez-Beltrán J, Rodríguez-Martínez JM, Costas C, Aznar J, Pascual Á, Blázquez J. Urinary tract physiological conditions promote ciprofloxacin resistance in low-level-quinolone-resistant *Escherichia coli.* Antimicrob Agents Chemother 2016; 60: 4252-8

We try to understand the genetic mechanisms involved in genome stability in bacteria, and their roles in evolution and adaptation. Specifically, we study both stable and induced hypermutation/hyper-recombination as "bacterial strategies" to speed adaptation to environmental stress. This knowledge could be useful i) to prevent development of antibiotic resistance in bacterial pathogens (including *Escherichia coli*, *Pseudomonas aeruginosa*, or *Mycobacterium tuberculosis*) and ii) to improve prokaryotic species of industrial interest (such as *Streptomyces*, *Bifidobacterium, Rhodococcus, Corynebacterium, Pyrococcus*, and *Halobacterium*).

Main research lines:

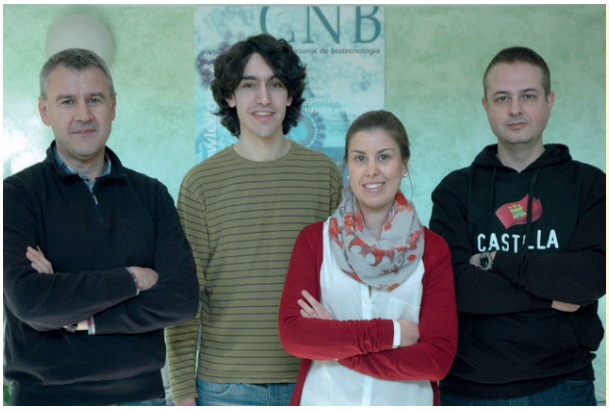
1. Regulation of hypermutation and hyper-recombination
   - Transcriptional regulation of specialized DNA polymerases (SOS regulon)
   - Regulation of mismatch repair
   - Effect of antibiotics on bacterial mutation and recombination: antibiotics as promoters of antibiotic resistance
2. Genetic basis of genome stability in prokaryotes lacking a canonical MutS-MutL-based DNA mismatch repair (MMR) system. We are studying possible alternative MMR pathways in these organisms, including most Actinobacteria (*Mycobacterium, Streptomyces, Rhodococcus*) and some Archaea (*Pyrococcus, Halobacterium*).
3. Development of hypermutant/hyper-recombinant prokaryotes of industrial interest as biotechnological tools to produce improved biosynthetic or degrading pathways. We also explore the possibility of developing genetically super-stable strains.
4. Preventing emergence and evolution of antibiotic resistance, by searching for and characterizing compounds able to inhibit the development of antibiotic resistance (by mutation, recombination and horizontal transfer).

1



1

A *Mycobacterium* strain in which the *nucS* gene was eliminated (1) produces a large number of mutants resistant to the antibiotic in the Petri dish (rifampicin), whereas the parental strain (with active *nucS*) (2) produces many fewer mutants (A Castañeda & J Blázquez).



Cell cycle, DNA replication, and genome instability in eukaryotes

GROUP LEADER
Arturo Calzada

PHD STUDENTS
Fernando Devesa
Fábia Gomes

TECHNICIAN
Ángel Valera

MASTER'S STUDENT
Arantxa Agote

UNDERGRADUATE STUDENTS
Pablo Aza
Débora Cavero

SELECTED PUBLICATIONS

Calzada A. Choice of Origins and Replication Timing Control in Budding Yeast. In: The Initiation of DNA Replication in Eukaryotes. Kaplan DL, editor. Springer. 2016. pp. 23–38.

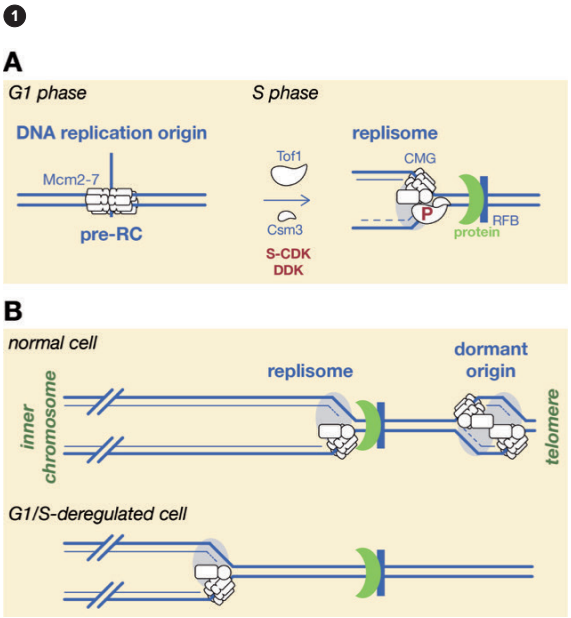
Lombraña R, Álvarez A, Fernández-Justel JM, Almeida R, Poza-Carrión C, Gomes F, Calzada A, Requena JM, Gómez M. Transcriptionally Driven DNA Replication Program of the Human Parasite Leishmania major. Cell Rep 2016; 16: 1774–86

We aim to understand how eukaryotic cells duplicate chromosomes during cell division, and regulate precise, complete synthesis of every DNA segment so that progeny inherit stable genomes. This process normally concludes successfully each of the many divisions that generate cell populations and pluricellular individuals. Cells do so despite the many difficulties that arise, including the brief period available to replicate very large genomes, impediments to fork progression posed by chromatin constraints or DNA-damaging insults, spatial concurrence with processes such as transcription, DNA repair or chromosome compaction, and maintenance of synchrony with mitosis. Spontaneous chromosome replication aberrations are linked to human diseases like cancer, which highlights the relevance of understanding how robust function of DNA replication machinery is regulated.

During these two years, we focused on the regulation of Tof1 in the budding yeast *Saccharomyces cerevisiae*. Tof1 binds Csm3 and the CMG DNA-replication helicase at yeast replisomes, as do their homologues in other eukaryotes, to regulate fork progression, especially through protein-DNA replication barriers (Figure 1A). Whether Tof1 binding to Csm3, recruitment to replisomes, activation/inhibition or function are directly regulated during the cell cycle, remains unknown in any eukaryote. We found that the Tof1/Csm3 complex is regulated by phosphorylation of Tof1 by specific cell cycle and DNA replication kinases throughout the cell cycle.

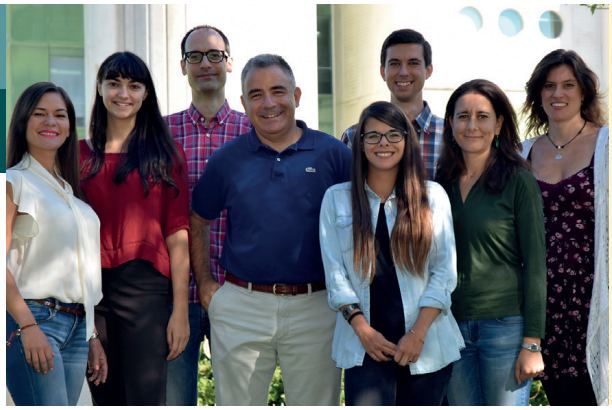
We also studied the regulation of specification and choice of DNA replication origins, still poorly understood, where multiple determinants define origin subtypes and make the purpose of this organization unclear. Strong correlations suggest that insufficient origin activation generates chromosome instability in early oncogenesis, although the mechanism involved is uncertain. In budding yeast cells, we modelled oncogenic deregulation of the G1/S transition (the most frequent mutation in human cancer cells) to study whether spontaneous origin insufficiency directly drives chromosome instability, and how this occurs (Figure 1B).

1



1

Recent results on the regulation of fork progression and origin usage for completion of eukaryotic chromosome replication and genome stability. (A) The Tof1/Csm3 complex, recruited to licensed DNA replication origins to form part of the replisome, is regulated by phosphorylation of Tof1 dependent on S-phase kinases. (B) G1/S deregulation, present in most cancer cells, deprives origin redundancy at specific chromosome regions by insufficient firing of early and dormant origins in yeast. As a result, contrary to normal cells (top) delayed replication progression and under-replication enrich at those regions (bottom) leading to elevated rates of chromosome instability.



Bacterial engineering for biomedical applications

GROUP LEADER

Luis Ángel Fernández

POSTDOCTORAL SCIENTIST

Beatriz Álvarez González

PHD STUDENTS

Massiel Cepeda Molero
Carmen Mañas Torres
David Ruano Gallego
Lidia Cerdán García
Alejandro Asensio Calavia
Eva Pico Sánchez

TECHNICIAN

Yago Margolles

MASTER'S STUDENTS

Lidia Cerdán García
Álvaro Ceballos Munuera

SELECTED PUBLICATIONS

Bodelón G, Marín E, Fernandez LA. Analyzing the role of periplasmic folding factors in the biogenesis of OMPs and members of the type V secretion system. Methods Mol Biol 2015; 1329: 77-110

Ruano-Gallego D, Álvarez B, Fernández LA. Engineering the controlled assembly of filamentous injectisomes in *E. coli* K-12 for protein translocation into mammalian cells. ACS Synth Biol 2015; 4: 1030-1041

Piñero-Lambea C, Ruano-Gallego D, Fernández LA. Engineered bacteria as therapeutic agents. Curr Opin Biotechnol 2015; 35: 94-102.

Salema V, López-Guajardo A, Gutiérrez C, Mencía M, Fernández LA. Characterization of nanobodies binding human Fibrinogen selected by *E. coli* display. J Biotechnol 2016; 235: 58-65

Salema V, Mañas C, Cerdán L, Piñero-Lambea C, Marín E, Roovers R, van Bergen en Henegouwen PM, Fernández LA. High affinity nanobodies against human Epidermal Growth Factor Receptor selected on cells by *E. coli* display. MAbs 2016; 8: 1286-1301

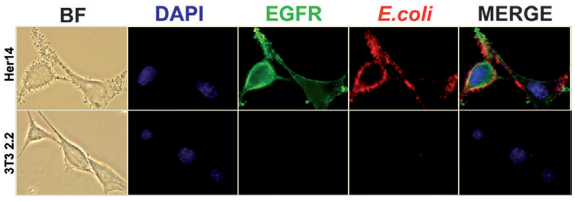
Our research is aimed at engineering bacteria for biomedical applications, including selection of small recombinant antibodies and design of bacteria for diagnostic and therapeutic use *in vivo*. We study protein secretion systems in pathogenic *Escherichia coli* strains and engineer them to develop protein nanomachines that can be applied for selection of recombinant antibodies and delivery of therapeutic proteins by non-pathogenic *E. coli* strains. We use single-domain antibodies (sdAb) from camelid V_{HH} domains, termed nanobodies; these are the smallest known antibody fragments with full antigen-binding capacity. We use synthetic biology approaches and genome engineering to combine expression of these modular parts in the bacteria designed.

Our major achievements in these two years are:

Development of *E. coli* display for selection of nanobodies that bind tumour cell surface antigens and generate "synthetic adhesins". We previously developed an *E. coli* system for nanobody library display and selection of high affinity binders to purified protein antigens. We expanded this technology and demonstrated its potential for isolation of nanobodies to tumour cell surface antigens such as human epidermal growth factor receptor (EGFR). We successfully employed *E. coli* display for selection with recombinant EGFR-Fc protein and direct screening of live EGFR-expressing tumour cell lines. These nanobodies are the basis of "synthetic adhesin" constructs that can drive attachment of engineered bacteria to tumour cells that express cell surface antigens.

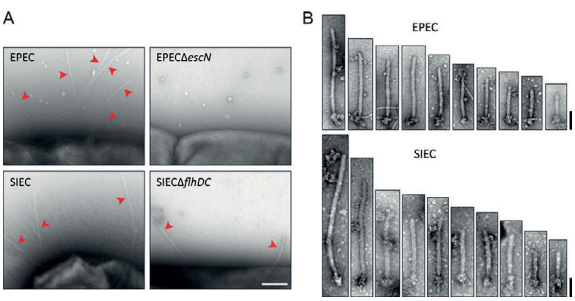
Generation of a synthetic injector *E. coli* strain (SIEC) for protein translocation into human cells. We intend to use the molecular syringes (injectisomes), encoded by the type III protein secretion system (T3SS) from enteropathogenic *E. coli* (EPEC), to deliver therapeutic nanobodies from *E. coli* into cytosol of human cells. We engineered expression of EPEC injectisomes in the non-pathogenic *E. coli* K-12 strain, which allows us to specifically deliver a protein of interest in mammalian cell cytosol. *E. coli* injection of proteins does not require bacterial invasion of the eukaryotic cell or transfer of any genetic material.

1



1 Bright field (BF) and fluorescence micrographs showing *E. coli* (red) displaying nanobodies that bind to human EGFR (green) expressed on the surface of the Her14 cell line. Note that these same bacteria do not bind the isogenic control cell line 3T32.2, which does not express human EGFR. Nuclei and bacteria are DNA-stained (blue) (from Salema *et al.*, 2016).

2



2 SIEC injectisomes seen by transmission electron microscopy (TEM). (A) TEM micrographs of negatively stained bacteria of the indicated strains. Injectisome filaments are labelled (red arrowheads). Scale bar, 200 nm. (B) TEM micrographs showing representative injectisome-like particles after purification from induced EPEC and SIEC bacteria. Injectisome particles are aligned at their basal bodies to show their filament lengths. Scale bars, 50 nm (from Ruano-Gallego *et al.*, 2015).

Intracellular bacterial pathogens

GROUP LEADER

Francisco García del Portillo

SENIOR SCIENTIST

María Graciela Pucciarelli

POSTDOCTORAL SCIENTIST

Sónia Castanheira

PHD STUDENTS

Juan José Cestero
Alberto Díaz
Gadea Rico-Pérez
Estel Ramos-Marquès

TECHNICIANS

Pablo García
Rocio Benavente

VISITING PHD STUDENTS

Damián Lobato-Marquéz
(CIB-CSIC, Madrid, Spain)
Nicolás Figueroa
(Inst de Biología Molecular CONICET, Rosario, Argentina)

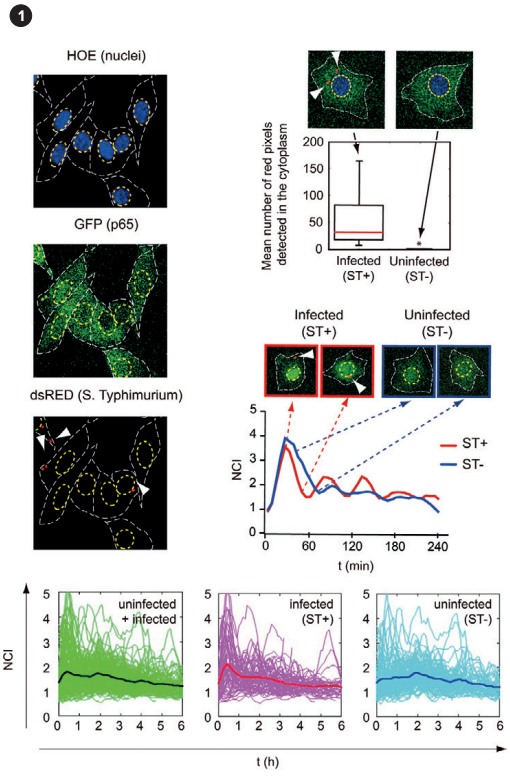
MASTER'S STUDENTS

Luis Armando Hernández
Cristina Arévalo

Our laboratory aims to understand the physiological changes undergone by intracellular bacterial pathogens such as *Salmonella* and *Listeria* when they colonize the intracellular niche of eukaryotic cells.

In *Salmonella*, we are interested in deciphering the structural changes that take place in the main cell wall component, peptidoglycan (PG), when the pathogen persists inside the host cell. We are also identifying elements of the enzymatic machinery involved in PG synthesis, remodelling and recycling pathways that are regulated when the pathogen adapts to the intracellular lifestyle. This information is used to determine how these changes affect innate immune host defences involving intracellular receptors of the nucleotide-binding oligomerization domain (NOD)-like receptor (NLR) family. Our laboratory is also developing new studies based on single-cell and real-time imaging technologies. These approaches are clarifying new routes of communication between intracellular *Salmonella* and the autophagy machinery. We are also testing whether *Salmonella*-infected cells alter the physiology of uninfected bystander cells.

Our interest in *Listeria moocytogenes* focuses on the large family (>40 members) of surface proteins covalently bound to the peptidoglycan and that bear an LPXTG-anchoring motif. The abundance of this protein class remains an enigmatic feature of *Listeria* biology. We intend to define the profile of these surface proteins in infection-related conditions, including the intracellular environment of eukaryotic cells and blood. Surface proteins are subsequently analysed to decipher the underlying regulatory mechanisms. Given the capacity of *Listeria* to grow in the cold, we are currently studying how this pathogen readjusts surface protein content following cold adaptation. Our data point to regulatory mechanisms that could upregulate production of several surface proteins at low temperatures. This information is currently being processed for application in the food industry to control *Listeria* proliferation.



1

Monitoring by single-cell analyses of NF-kappaB translocation dynamics (cytosol-nucleus) in fibroblasts exposed to *Salmonella*

SELECTED PUBLICATIONS

Lobato-Márquez D, Moreno-Córdoba I, Figueroa V, Díaz-Orejas R, García-del Portillo F. Distinct type I and type II toxin-antitoxin modules control Salmonella lifestyle inside eukaryotic cells. Sci Rep 2015; 5: 9374

López-Montero N, Ramos-Marquès E, Risco C, García-Del Portillo F. Intracellular Salmonella induces aggrephagy of host endomembranes in persistent infections. Autophagy 2016; 12: 1886-1901

Ramos-Marquès E, Zambrano S, Tiérrez A, Bianchi ME, Agresti A, García-del Portillo, F. Single-cell analyses reveal an attenuated NF-kappaB response in the Salmonella-infected fibroblast. Virulence 2016; 30: 1-22

Rico-Pérez G, Pezza A, Pucciarelli MG, de Pedro MA, Soncini FC, García-del Portillo F. A novel peptidoglycan D,L-endopeptidase induced by Salmonella inside eukaryotic cells contributes to virulence. Mol Microbiol 2016; 99: 546–556

Quereda JJ, García-del Portillo F, Puciarelli MG. *Listeria monocytogenes* remodels the cell surface in the blood-stage. Environ Microbiol Rep 2016; 8: 641–648

Molecular infection biology

GROUP LEADER

Daniel López

POSTDOCTORAL SCIENTISTS

Elvira Marin
Esther García
Mar Cordero
Ilaria Peschiera
Marta Ukleja

PHD STUDENTS

Lara Kricks
Rabea Wagner
Ivan Acosta
Diana Damián
Juan Carlos García
(University of Würzburg, Germany)
Benjamin Mielich-Süß
(University of Würzburg, Germany)
Charlotte Wermser
(University of Würzburg, Germany)

TECHNICIAN

Carmen Ortega

MASTER'S STUDENTS

Javier de la Fuente
Aida Mayorgas

SELECTED PUBLICATIONS

Bramkamp M, Lopez D. Exploring the existence of lipid rafts in bacteria. Microbiol Mol Biol Rev 2015; 79: 81-100

Schneider J, Klein T, Mielich-Süss B, Koch G, Franke C, Kuipers OP, Kovács AT, Sauer M, Lopez D. Spatio-temporal remodeling of functional membrane microdomains organizes the signaling networks of a bacterium. PLoS Genet 2015; 11: e1005140

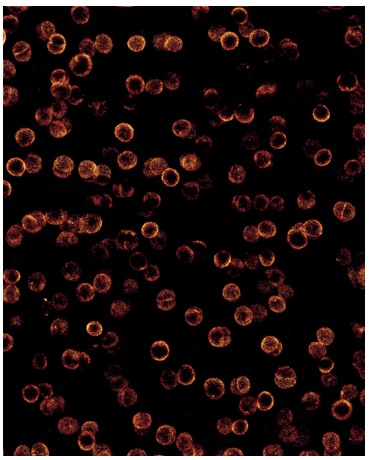
Schneider J, Mielich-Süss B, Böhme R, Lopez D. *In vivo* characterization of the scaffold activity of flotillin on the membrane kinase KinC of *Bacillus subtilis* Microbiology. 2015; 161: 1871-87

Eukaryotic membranes organize signal transduction proteins into microdomains, or lipid rafts, that are enriched in specific lipids such as cholesterol. Lipid rafts are important for the correct activity of numerous cell functions, and their disruption causes serious defects in several signal transduction processes. The assembly of lipid rafts in eukaryotes has been considered a fundamental step during the evolution of cellular complexity, which suggests that prokaryotic organisms were too simple to require such sophisticated organization of their signalling networks.

My group discovered that bacteria organize many signal transduction processes in functional membrane microdomains constituted by specific lipids. Bacterial membranes contain lipid rafts similar to those found in eukaryotic cells. Perturbation of bacterial lipid rafts leads inevitably to strong, simultaneous impairment of all raft-harboured signal transduction pathways, which causes potent inhibition of infective potential in pathogenic bacteria.

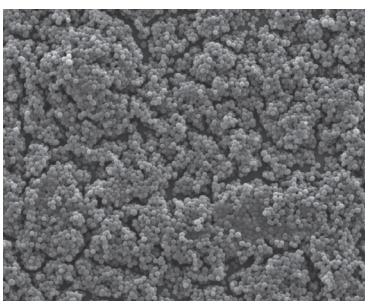
The discovery of lipid rafts in bacteria is a new concept in biology that we address in my laboratory. We use the human pathogen *Staphylococcus aureus* as a working model to study bacterial lipid rafts and to understand the structural components involved in their assembly and maintenance. The biological role of bacterial lipid rafts in regulating infection-related processes and the feasibility of targeting the integrity of these lipid rafts is a new strategy for anti-microbial therapy.

❶



❷

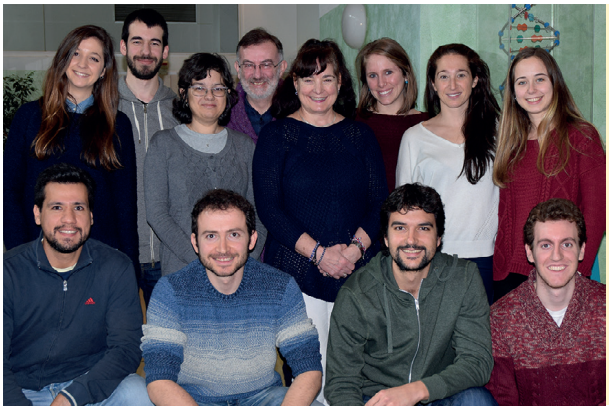
❸



❶ Super-resolution dSTORM fluorescence microscopy image of *Staphylococcus aureus* cells

❷ *Staphylococcus aureus* colony growing in a stress environment. The bacterial population diversifies and generates sectors of mutants with distinct physiology to respond to stress

❸ Scanning electron microscopy image of a *S. aureus* biofilm growing on a glass plate



Opportunistic pathogens

GROUP LEADER

José Luis Martínez

POSTDOCTORAL SCIENTISTS

Blanca Sánchez
Sara Hernando-Amado
Aida Pitarch
Felipe Lira

PHD STUDENTS

Fernando Corona
Manuel Alcalde
Paula Blanco
Fernando Sanz-García

TECHNICIAN

Trinidad Cuesta

MASTER'S STUDENTS

Teresa Gil
Cristina Rosco
Alejandro Tejada

VISITING SCIENTIST

Pilar Muñoz
(Universidad Europea de Madrid)

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 two years:

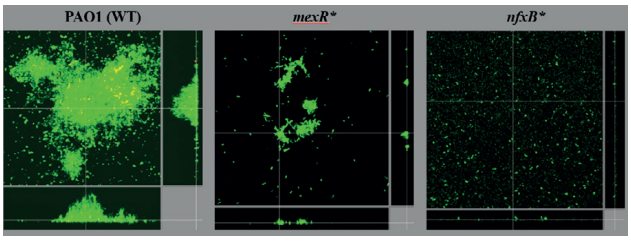
a) We proposed that bacterial metabolism and antibiotic resistance are closely interlinked, and have explored the contribution of two global regulators of antibiotic resistance and virulence: the post-transcriptional regulator of carbon metabolism from *Pseudomonas aeruginosa* (Crc), and the RNAse G from *Stenotrophomonas maltophilia*. Our results support the idea that intrinsic resistance to antibiotics is not merely an adaptive response to presence of the antimicrobial, but an emergent property linked to the bacterial metabolic activity needed to maintain cell homeostasis.

b) We proposed several rules for predicting the emergence of antibiotic resistance and are currently standardizing these tools, which are based on experimental evolution, whole-genome sequencing and functional assays. Using this approach, we characterized mechanisms of resistance to latest-generation antibiotics and identified the target and the mechanisms of resistance to antibiotics still being developed.

c) 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 deterministic (mainly populational) and stochastic aspects (at the single cell level) of antibiotic resistance.

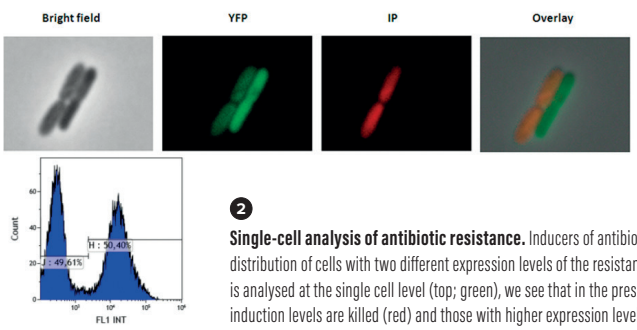
d) Microbial signalling is a complex situation with at least two levels of complexity. Some systems, such as the stringent response, operate mainly at the single cell level because the signal is not exported, whereas others such as the quorum sensing response, in which the signal is exported, operate at the population level. We aim to analyse the hierarchical integration of these signalling networks and study how acquisition of antibiotic resistance affects bacterial signalling, both at the single cell and at the population level.

1



1 **Effect of acquiring resistance on bacterial physiology at the population level.** Mutations leading to antibiotic resistance (*mexR** and *nfxB**) reduce the ability of *Pseudomonas aeruginosa* to form biofilm (wild-type PA01).

2



2 **Single-cell analysis of antibiotic resistance.** Inducers of antibiotic resistance can produce a discrete distribution of cells with two different expression levels of the resistance determinant (bottom). When expression is analysed at the single cell level (top; green), we see that in the presence of antibiotics, cells with lower induction levels are killed (red) and those with higher expression levels of the resistance determinant survive.

SELECTED PUBLICATIONS

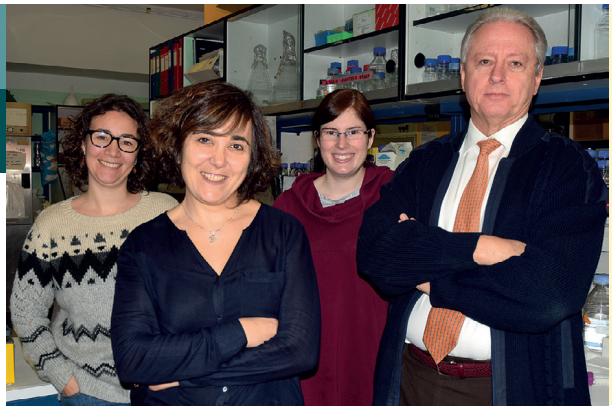
Martínez JL, Coque T, Baquero F. What is a Resistance Gene? Ranking Risks on Resistomes. Nat Rev Microbiol 2015; 13: 116-23

Berendonk TU, Manaia CM, Merlin C, Fatta-Kassinos D, Cytryn E, Walsh F, Bürgmann H, Sørum H, Norström M, Pons MN, Kreuzinger N, Huovinen P, Stefani S, Schwartz T, Kisand V, Baquero F, Martinez JL. Tackling antibiotic resistance: the environmental framework. Nat Rev Microbiol 2015; 13: 310-317

Reales-Calderón JA, Corona F, Monteoliva L, Gil C, Martínez JL. Quantitative proteomics unravels that the post-transcriptional regulator Crc modulates the generation of vesicles and secreted virulence determinants of *Pseudomonas aeruginosa*. J Proteomics 2015; 127: 352-364

Bernardini A, Corona F, Dias R, Sánchez MB, Martínez JL. The inactivation of RNase G reduces the *Stenotrophomonas maltophilia* susceptibility to quinolones by triggering the heat shock response. Front Microbiol 2015; 6: 1068

Sanchez MB, Martínez JL. Regulation of Sm*qnr* expression by SmqnrR is strain-specific in *Stenotrophomonas maltophilia*. J Antimicrob Chemother 2015; 70: 2913-2914



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

GROUP LEADER

Rafael P. Mellado

SENIOR SCIENTIST:

Sonia Gullón

POSTDOCTORAL SCIENTIST

Rebeca López Vicente

TECHNICIAN

Silvia Marín

SELECTED PUBLICATIONS

Gullón S, Marín S, Mellado RP. Overproduction of a model Sec- and Tat-Dependent secretory protein elicits different cellular responses in *Streptomyces lividans*. PLoS One 2015; 10: e0133645

Gullón S, Vicente RL, Valverde JR, Marín S, Mellado RP. Exploring the feasibility of the Sec route to secrete proteins using the Tat route in *Streptomyces lividans*. Mol Biotechnol 2015; 57: 931-938

Valverde JR, Gullón S, Mellado RP. Looking for rhizobacterial ecological indicators in agricultural soils using 16S rRNA metagenomic amplicon data. PLoS One 2016; 11: e0165204

Vicente RL, Gullón S, Marin S, Mellado RP. The three *Streptomyces lividans* HtrA-like proteases involved in the secretion stress response act in a cooperative manner. PLoS One 2016; 11: e0168112

The group has long-standing interest in the physiological and molecular characterization 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 to degrade misfolded proteins in *Streptomyces*.

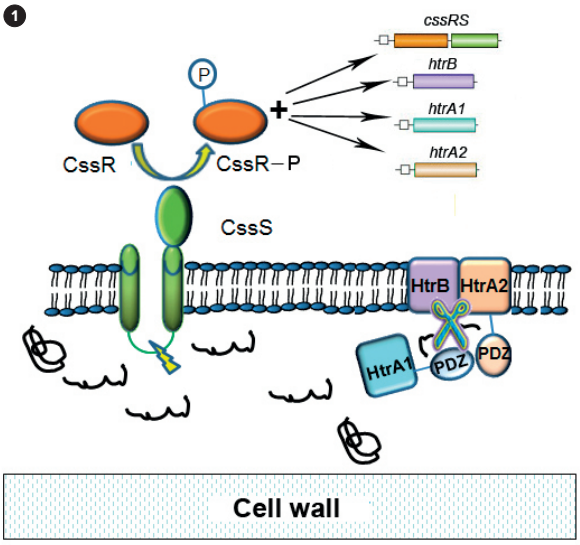
When secretory proteins are overproduced in *Streptomyces*, the CssRS two-component system mediates the so-called secretion stress response, inducing expression of three specific HtrA-like protease genes. CssS (sensor) or CssR (regulator) deficiencies affect the transcriptional levels of the three genes that encode the HtrA-like proteins.

The *in vitro* phosphorylated purified His_6-CssR binds to the respective regulatory regions of the HtrA-like protease genes and to that of its own gene, as determined by gel retardation assays. Deficiencies in the sensor or regulator coding genes leads to production of inactive extracellular alpha amylase, as occurs when any of the three protease genes is inactivated, which we determined by Western blot assays. Propagation of each of the protease genes in single copy does rescue the respective mutation, but there is no cross-complementation among the different protease individual mutants. Propagation at high copy number of any of the protease genes causes indiscriminate degradation of extracellular alpha-amylase. The three proteases must be functional simultaneously to ensure efficient degradation of misfolded secretory proteins.

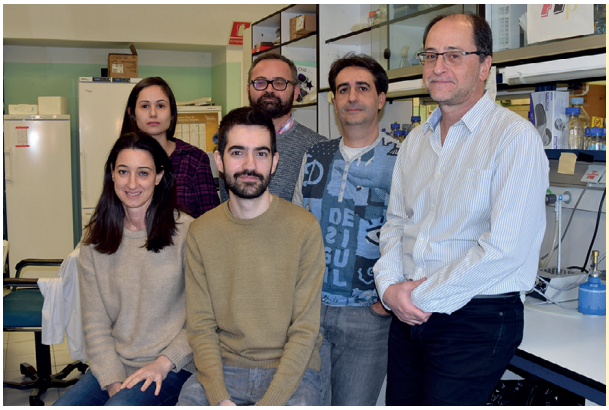
These findings are of particular relevance with respect to characterizing potential bottlenecks in *S. lividans* secretion and optimizing *S. lividans* for the overproduction of secretory proteins of industrial application.

Our research line monitoring rhizobacterial communities, using 16S rRNA metagenomic amplicon data, allowed us to determine that *Actinomycetes* are ubiquitous and suitable as meaningful ecological indicators in agricultural soils.

1



1 Extracellular accumulation of misfolded secretory proteins induces the CssR-S two-component system, triggering synthesis of the three specific HtrA-like proteases (HtrA, HtrA2 and HtrB).



Regulation of the metabolism of hydrocarbons in bacteria

GROUP LEADER
Fernando Rojo

SENIOR SCIENTIST
Renata Moreno

POSTDOCTORAL SCIENTISTS
Emma Sevilla
Sofía Hernández

PHD STUDENTS
Ruggero La Rosa
Dione Sánchez

TECHNICIAN
Luis Yuste

VISITING SCIENTIST
Elva Quiroz-Rocha
(Inst de Biotecnología, Universidad Nacional Autónoma de Mexico)

UNDERGRADUATE STUDENT
Guillermo Gómez

SELECTED PUBLICATIONS

Moreno R, Hernández-Arranz S, La Rosa R, Yuste L, Madhushani A, Shingler V, Rojo F. The Crc and Hfq proteins of *Pseudomonas putida* co-operate in catabolite repression and formation of ribonucleic acid complexes with specific target motifs. Environ Microbiol 2015; 17: 105-118

La Rosa R, Nogales J, Rojo F. The Crc/CrcZ-CrcY global regulatory system helps the integration of gluconeogenic and glycolytic metabolism in *Pseudomonas putida*. Environ Microbiol 2015; 17: 3362-3378

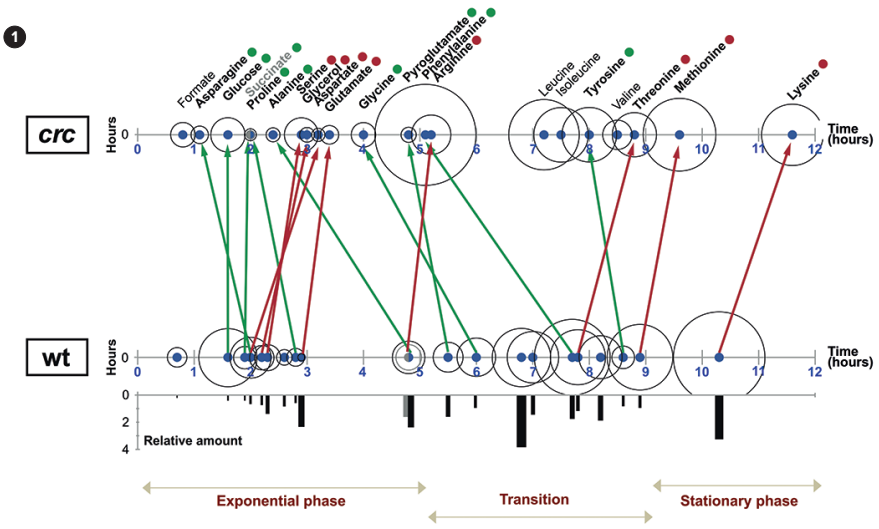
Sevilla E, Yuste L, Rojo F. Marine hydrocarbonoclastic bacteria as whole-cell biosensors for n-alkanes. Microb Biotechnol 2015; 8: 693-706

La Rosa R, Behrends V, Williams HD, Bundy JG, Rojo F. Influence of the Crc regulator on the hierarchical use of carbon sources from a complete medium in Pseudomonas. Environ Microbiol 2016; 18: 807-818

Hernández-Arranz S, Sánchez-Hevia D, Rojo F, Moreno R. Effect of Crc and Hfq proteins on the transcription, processing and stability of the *Pseudomonas putida* CrcZ sRNA. RNA 2016; 22: 1902-1917

To be competitive in the environments they colonize, bacteria must optimize 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 compound is consumed. This selection implies a complex regulatory process termed catabolite repression. Unravelling the molecular mechanisms that underlie these regulatory events helps us comprehend how bacteria coordinate their metabolism and gene expression programmes and optimize growth. It also aids in the design and optimization 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 compounds.

The regulatory proteins and molecular mechanisms responsible for catabolite repression differ among microorganisms. Our work focuses on *Pseudomonas putida,* a bacterium that has a very versatile metabolism, colonizes 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 termed CrcZ and CrcY, the levels of which vary greatly depending on growth conditions, antagonize the inhibitory effect of Hfq and Crc. We aim to characterize the influence of Crc, Hfq, CrcZ and CrcY on *P. putida* physiology, the signals to which they respond, and the molecular mechanisms by which they regulate gene expression. We intend to determine how they modulate metabolism in response to fluctuating environmental conditions.



1

Influence of the Crc regulatory protein on the hierarchy of assimilation of the compounds in complete medium in a 12-hour culture. Blue dots, time at which compound concentration decreases by 50%. Circles, speed of assimilation of each compound; smaller diameter means more rapid assimilation. wt, *Pseudomonas putida* KT2440; *crc*, mutant derivative lacking the *crc* gene.

Genetic control of the cell cycle

GROUP LEADER

Miguel Vicente

POSTDOCTORAL SCIENTISTS

Susanne Gola
Ana Isabel Rico
Alicia Sánchez-Gorostiaga

PHD STUDENTS

Laura Cueto
Marcin Krupka
Cristina Ortiz

TECHNICIANS

Marina Cabrerizo
Mercedes Casanova
Pilar Palacios

VISITING SCIENTIST

Derly Andrade
(Pontificia Universidad Católica de Chile)

MASTER'S STUDENT

Adrián Izquierdo

SELECTED PUBLICATIONS

Cabré EJ, Monterroso B, Alfonso C, Sánchez-Gorostiaga A, Reija B, Jiménez M, Vicente M*, Zorrilla S*, Rivas G*. The nucleoid occlusion SlmA protein accelerates the disassembly of the FtsZ polymers without affecting their GTPase activity. PLoS One 2015; 10: e0126434 (*equal contribution)

Gola S, Munder T, Casonato S, Manganelli R. Vicente M. The essential role of SepF in mycobacterial division. Mol Microbiol 2015; 97: 560–576

Ortiz C*, Kureisaite-Ciziene D*, Schmit F, McLaughlin SH, Vicente M, Löwe J. Crystal structure of the Z-ring associated cell division protein ZapC from Escherichia coli. FEBS Lett 2015; 589: 3822–3828 (*equal contribution)

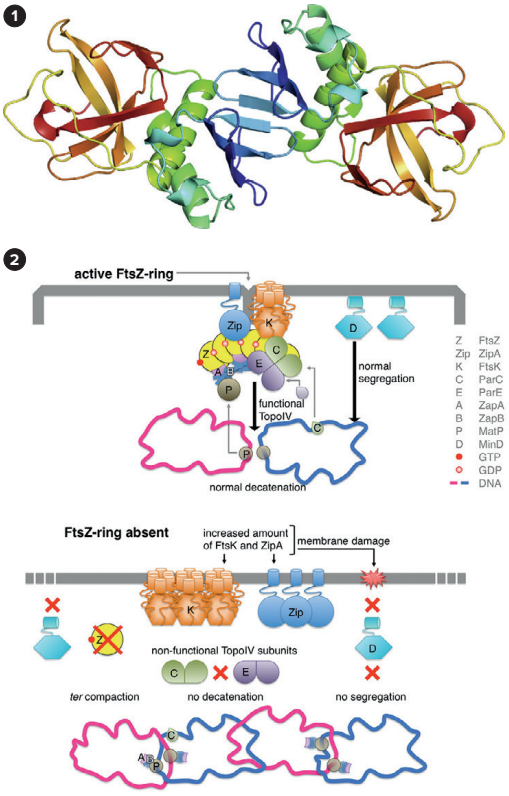
Ortiz C, Natale P, Cueto L, Vicente M. The Keepers of the Ring: regulators of FtsZ assembly. FEMS Microbiol Rev 2016; 40: 57-67

Sánchez-Gorostiaga A, Palacios P, Martínez-Arteaga R, Sánchez M, Casanova M, Vicente M. Life without division: Physiology of *Escherichia coli* FtsZ-deprived filaments. mBio 2016; 7: e01620-16

FtsZ, a GTPase found in the cytoplasm of most bacteria, is the major component of the machinery responsible for division (the divisome) in *Escherichia coli.* As well as forming polymers, it interacts with additional proteins that contribute to its function by forming a ring at the midcell that is essential to constrict the membrane. FtsZ is anchored indirectly to the membrane and is prevented from polymerizing at locations where septation is undesired. Several FtsZ properties are mediated by other proteins that function as keepers of the ring. ZipA and FtsA serve to anchor the ring, and together with a set of Zap proteins, they stabilize it. The MinCDE and SlmA proteins prevent polymerization of FtsZ at sites other than the midcell. ClpP degrades FtsZ, an action prevented by ZipA. Many of the FtsZ keepers interact with FtsZ through a central hub located at its carboxy terminal end.

Besides causing filamentation due to division arrest, the very low FtsZ levels in FtsZ-deprived cells have severe pleiotropic effects on *E. coli* physiology that compromise bacterial survival. In contrast to FtsZ-deprived cells, the viability of filaments formed by a conditional mutant is not affected when FtsZ activity is lost, because the amount of the protein remains unperturbed. We propose that the quest for new antimicrobials that target FtsZ should be directed to decreasing the number of FtsZ molecules rather than to inhibiting its activity.

The *Mycobacteria* include important human pathogens. The mycobacterial SepF homologue is highly conserved in the *Mycobacterium tuberculosis* complex. SepF is a key component of the mycobacterial divisome, necessary for division. It interacts with FtsZ and is located in a ring-like structure at potential division sites in an FtsZ-dependent manner, making it an attractive target for proliferation blockade.



1 Structure of one of the keepers of the ring, the ZapC dimer (from Ortiz *et al.*, 2015).

2 The chaos produced in vital bacterial machineries by FtsZ deprivation (bottom), compared to their state in normal conditions (top) (from Sánchez-Gorostiaga *et al.*, 2016).