# Celebrating the career and legacy of Professor Pascale Cossart

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# EDITORIAL: CELEBRATING THE CAREER AND LEGACY OF PROFESSOR PASCALE COSSART

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It is an enormous pleasure and honour for me to contribute this editorial to introduce the special issue that we have assembled to honour the numerous, unique and outstanding scientific achievements of Pascale Cossart and her group. Pascale Cossart, a distinguished Professor at the Institut Pasteur in Paris, France, has been a worldwide leader in the study of fundamental processes in molecular and cellular microbiology and host pathogen interactions for more than 30 years. Using mainly *Listeria monocytogenes,* but also *Ricketssia conorii,* as models she has made several seminal findings in the field of infection biology, cell biology and epigenetics. Her work on fundamental RNA biology and bacterial regulation, and more recently the discovery of a new antibiotic resistance mechanism that is called ribosome splitting, are examples of her elegant contributions to the field.

In this Special issue of *Molecular Microbiology*, colleagues, former students, postdoctoral fellows and friends of Pascale Cossart have gathered together to contribute reviews and articles in the area of her major research interests. The special issue starts with a personal tribute to Pascale Cossart, contributed by *Werner Goebel*, a longstanding colleague and competitor who traces many of the ground-breaking findings made by Pascale Cossart and her group.

*Listeria monocytogenes* is reported in all textbooks as an intracellular pathogen that replicates in the cytosol of human cells after its escape from the vacuole. However, recently this paradigm was challenged by observations that *L. monocytogenes* can also adopt a vacuolar lifestyle when persisting within cells (Kortebi *et al.*, 2017). As another example, *Mycobacterium tuberculosis,* which was thought for a long time to be a strictly intra-vacuolar pathogen, was recently shown to rupture the phagosome and to contact the cytosol of the phagocytes it infects (Russell, 2016, Simeone *et al.*, 2009). This discovery was very important to better understand former unsolved steps of the disease process, as it is associated with altered intracellular signalling. In particular, this knowledge is important for the development

of vaccines, a priority for tuberculosis research (Groschel *et al.*, 2017). Thus a strict distinction between vacuolar and cytosolic intracellular pathogens is not evident. Similarly, the strict distinction between extracellular and intracellular pathogens does not hold true anymore. In a very interesting opinion article *Arturo Casadevall and Ferric Fang* show that new findings blur the boundaries between extracellular and intracellular pathogens and they discuss the benefits and caveats arising from the intracellular pathogen concept.

Today, multidrug resistant bacteria are a worldwide public health threat with increasing mortality and tremendous costs. Thus multidisciplinary research efforts are of utmost importance to better understand resistance emergence, persistence, dissemination and to find new, effective drugs. In an illustration of how the knowledge of ancient civilisations might help to solve this modern problem, *Julian Davis* and *Shekooh Behroozian* provide insights on how to use native environmental resources for extraction of natural products, so called medicinals. In particular, they present a personal view on the antimicrobial properties of clay minerals and propose that these underexplored natural resources may provide an alternative means to fight pathogens resistant to commonly used drugs.

Another fascinating approach to fight antibiotic resistance is proposed by *Jörg Vogel* who provides new ideas on how species-specific programmable RNA antibiotics could be developed. Indeed, broad-spectrum antibiotics are killing many bacteria present in and on our body, including both beneficial as well as pathogenic ones. Thus, the possibility of selective killing of pathogenic and/or multidrug resistant bacteria is very appealing, and might lead to microbiome editing for health. While proposing different ways to use short antisense oligonucleotides as antimicrobials, he also highlights the caveats including specificity, delivery, resistance and persistence.

Antibiotics are mainly seen as drugs to treat bacterial infections by inhibiting bacterial growth or killing them. However, as most antibiotics are naturally produced by soil organisms, their primary ecological function in the environment may be different. *Pishchany Gleb* and *Roberto Kolter* consider possible ecological roles of antibiotics and discuss their alternative functions at sub-inhibitory concentrations, such as the modulation of gene expression or biofilm formation, their influence on the stability of RNA molecules, and effects on bacterial evolution by increasing mutagenesis rates and horizontal gene transfer through conjugation. They argue that it is important to start studying the importance of these molecules in the

context of multispecies communities and in environmental conditions to understand how antibiotics might affect microbial communities and to expand our knowledge on these other, understudied effects of antibiotics.

*Fernando Baquero, Val Fernadez-Lanza, Melodie Duval* and *Teresa Coque* consider another idea that puts the environment into the focus of antibiotic resistance, namely the impact of gene – environment interactions on the acquisition of antibiotic resistance determinants, the ecogenetics of antibiotic resistance. As a specific example of how the ecology of an organism may shape the acquisition of antibiotic resistance, they examine *L. monocytogenes*, as it is intriguing that these bacteria have retained susceptibility to the key antimicrobials used to treat infections. This is even more surprising as *L. monocytogenes* is found ubiquitous in the environment and in a large spectrum of animals and plants. The authors discuss the impacts of global ecology, population size, genome structure, environmental stresses, and suggest that the many biocide and heavy metal resistance genes that are present in the *L. monocytogenes* genome may also play a role.

*Cameron Parson, Sangmi Lee* and *Sophia Kathariou* discuss these heavy metal resistance genes in *L. monocytogenes* and other Gram-positive bacteria further. They give a comprehensive update on the plasmid- and chromosomal-borne cadmium and arsenic resistance genes, and discuss their importance for environmental persistence. They suggest that the food-processing environment exerts a major selection pressure for cadmium resistance in *L. monocytogenes*, however many natural *Listeria* isolates are also highly resistant to several toxic metals. Thus, further studies will help to understand the ecological and evolutionary factors that drive acquisition, spread and diversification of the genomic elements conferring heavy metal resistance.

Two important new concepts in RNA biology have been discovered by the group of Pascale Cossart that are highlighted in the two following articles. *Pierre Mandin* and *Jörgen Johansson* give a short overview on how bacteria sense temperature changes and the impact this might have on virulence. For many years it was known that bacteria can sense temperature changes, and that in pathogenic bacteria virulence gene expression is often correlated with the transition from the environment (low temperature) to the human host (37°C). An example of temperature-regulated virulence gene expression the PrfA thermosensor of *L. monocytogenes* is described and compared to other bacterial

thermosensers. *Alejandro Toledo-Arana* and *Iñigo Lasa* discuss additional regulatory concepts by giving a masterly account of the advances made and new insights gained through the implementation of high-throughput methods for RNA profiling. Through genome-wide transcriptional profiling massive amount of antisense transcription, as well as conditional transcriptional termination and non-contiguous operons, have been discovered. Furthermore, the authors highlight riboswitch-dependent regulation of antisense RNA and overlapping transcription between neighbouring genes and present the related excludon concept, a new paradigm of regulation based on overlapping transcription.

Another rapidly expanding area of gene regulation studies was initiated by the discovery and characterization of small noncoding regulatory RNAs. Many of these control bacterial pathogenicity and the adaptation of bacteria to environmental stresses and to their hosts (Oliva *et al.*, 2015). *Jens Georg, David Lalaouna, Shengwei Hou, Steffen Lott, Isabelle Caldelari, Stefano Marzi, Wolfgang Hess* and *Pascale Romby* present a comprehensive and exciting overview on how targets of small RNAs can be identified, a still very challenging task. They show how by combining powerful computational approaches with experimental approaches the full extent of small RNA-depended regulatory networks can be elucidated.

Peptidoglycan is an essential molecule for bacteria that maintains their shape, cell integrity and provides a protective function against changing environmental conditions. Furthermore, peptidoglycan can be altered within the host to impair responses triggered by pattern recognition receptors (Pucciarelli & Garcia-del Portillo, 2018). Peptidoglycan is also studied as a target to control bacterial infections. However, these studies are rarely undertaken in natural environments but under laboratory conditions. Until now our knowledge on peptidoglycan stems mainly from few model bacteria, and intracellular bacteria have only been studied very recently. *Francisco Garcia-del-Portillo* provides an illuminating account of recent advances on probing peptidoglycan synthesis and assembly within eukaryotic host cells, with a particular focus on the enzymes involved in peptidoglycan metabolism in the context of symbiotic growth and persistent infection. He gives a detailed description of the enzymatic machinery identified mainly in *Escherichia coli* and of peptidoglycan recycling utilized by bacterial pathogens to evade immune defences. Furthermore, the current techniques that are used for analysis and isolation of cell wall muropeptides are described and examples of intracellular bacteria that have been analysed with these methods are summarised.

Peptidoglycan is also one of the three major constituents of the *L. monocyogenes* cell wall, along with anionic teichoic acid polymers and wall-associated and wall-anchored proteins. Polymeric teichoic acid is divided into wall teichoic-acid, which is directly conjugated to the peptidoglycan, and lipoteichoic acid, which is tethered to the plasma membrane. The *Listeria* teichoic acid polymers have important functions such as regulating bacterial growth, ion-homeostasis, biofilm formation, interaction with bacteriophages, host cell invasion and virulence. The *L. moncytogenes* serotyping scheme classifies strains into different serotypes. It recognizes structural and compositional changes on the cell wall surface and led to the discovery of strains belonging to specific serovars, which cause most human infections. In their MicroReview *Eric Sumrall, Anja Keller, Yang Shen* and *Martin Loessner* provide a comprehensive update on the synthesis, structural variability and function of teichoic acids in the genus *Listeria*. They discuss the impacts of teichoic acid decorations on serotype-specific designations, on phage resistance and virulence *via* wall-associated virulence proteins. They propose that the chemical synthesis of teichoic acids could be used for vaccine development. These engineered, teichoic acid-based vaccines could fully exploit their immunogenic potential and might represent a new strategy to fight infections with Gram-positive bacteria.

In the following article, *Eric Sumrall, Christopher Schefer, Jeanine Rismonod, Angelika Gründling, Martin Loessner* and *Yang Shen* present new research results on the function of teichoic acids in virulence of *L. monocytogenes.* They show that two different glycosyltransferases are required for the galactosylation of lipotechoic acid (LTA) and wall techioic acid (WTA), respectively. Thus *L. monocytogenes* encodes two surface-acting galactosyltransferases with distinct substrate specificities, which have an impact on the cell surface modification and InlB (an important virulence factor for invasion of eukaryotic cells) retention and function. The demonstration that modification of WTA alone is critical for InlB localization is interesting in that LTA and WTA are often referred to in the literature with minimal functional distinction. This study clearly indicates that these surface moieties have important functional differences, and leads one to wonder what other functions might be solely attributed to WTA *versus* LTA, or *vice versa*. Another important surface structure is pili. They have a pivotal role in the colonization of specific host tissues in many pathogenic bacteria. *Frederico Iovino, Priyanka Nannapaneni, Brigitta Henriques-Normark* and *Staffan Normark* provide a state-of-the art overview of the recent findings of type 1 pilus and its adhesive tip protein RrgA from *Streptococcus pneumoniae*. The type 1 pilus is an important

yet less studied surface associated virulence determinant of pneumococcal surface proteins, and thus the presentation of biochemical, structural and host pathogen interaction studies showing the impact of type 1 pilus in colonization and pathogenicity are very timely. Pneumococcal type 1 pili seem to confer a competitive advantage for nasopharyngeal colonization of humans, and the RrgA tip protein might be a good addition to the group of proteins used for the pneumococcal vaccine.

How did these different bacterial cell envelopes evolve? In particular, how did monoderm and diderm bacteria evolve? In the penultimate article of this issue *Daniela Megrian, Najwa Taib, Jerzy Witwinowski, Christophe Beloin* and *Simonetta Gribaldo* address the important question of cell envelope diversity across the bacterial domain. Based on the existence of diderm bacteria among the generally monoderm Firmicutes, the division of Gram-positive (monoderm) and Gram-negative (diderm bacteria) is discussed. Using an in depth and comprehensive phylogenomic analyses, together with results from a new experimental model, *Veillonella parvula*, a diderm Firmicute, the authors propose that the diderm envelope is ancestral within the Firmicutes and the monoderm envelope evolved multiple times through loss of the outer membrane. This provocative hypothesis will stimulate discussions and further studies to understand bacterial cell envelope evolution. Finally, *Megan de Ste Croix, Jonathan Holmes, Joseph Wanford, Richard Moxon, Marco Oggioni,* and *Christopher Bayliss* discuss the role of bottlenecks in the evolution of diversity-generating mechanisms of microbes during bacterial transmission and infection. They provide an authoritative summary of progress in the field and challenges for future studies.

This collection of articles reflects many of the areas of research to which Pascale Cossart has contributed, highlighting and building upon her outstanding findings over the last decades. We are confident that these contributions and ideas will inspire young researchers to embrace the upcoming challenges of the New Microbiology (Cossart, 2018).

**References**

Cossart, P., (2018) *The New Microbiology: From Microbiomes to CRISPR*. American Society of Microbiology.

Groschel, M.I., F. Sayes, S.J. Shin, W. Frigui, A. Pawlik, M. Orgeur, R. Canetti, N. Honore, R. Simeone, T.S. van der Werf, W. Bitter, S.N. Cho, L. Majlessi & R. Brosch, (2017) Recombinant BCG Expressing ESX-1 of *Mycobacterium marinum* Combines Low Virulence with Cytosolic Immune Signaling and Improved TB Protection. *Cell Rep* **18**: 2752-2765.

Kortebi, M., E. Milohanic, G. Mitchell, C. Pechoux, M.C. Prevost, P. Cossart & H. Bierne, (2017) *Listeria monocytogenes* switches from dissemination to persistence by adopting a vacuolar lifestyle in epithelial cells. *PLoS Pathog* **13**: e1006734.

Oliva, G., T. Sahr & C. Buchrieser, (2015) Small RNAs, 5' UTR elements and RNA-binding proteins in intracellular bacteria: impact on metabolism and virulence. *FEMS Microbiol Rev* **39**: 331-349.

Pucciarelli, M.G. & F. Garcia-del Portillo, (2018) Within-Host Envelope Remodelling and its Impact in Bacterial Pathogen Recognition. *Curr Issues Mol Biol* **25**: 43-60.

Russell, D.G., (2016) The ins and outs of the *Mycobacterium tuberculosis*-containing vacuole. *Cell Microbiol* **18**: 1065-1069.

Simeone, R., D. Bottai & R. Brosch, (2009) ESX/type VII secretion systems and their role in host-pathogen interaction. *Curr Opin Microbiol* **12**: 4-10.