



## INTER-ACADEMIES SYMPOSIUM

Académie des sciences, German National Academy of Sciences Leopoldina  
and The Royal Society

on

### “The New Microbiology”

May 14, 15 and 16, 2012

Institut de France  
23 quai de Conti  
Paris 6<sup>e</sup>

#### Organizing Committee

**Pascale Cossart** and **Philippe Sansonetti**,  
Académie des sciences,  
**Joerg Hacker** and **Jürgen Heesemann**,  
German National Academy of Sciences Leopoldina  
**David Holden** and **Richard Moxon**,  
The Royal Society

#### Organisation

Académie des sciences  
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## SCIENTIFIC PROGRAMME

**Monday, May 14<sup>th</sup> 2012 / Lundi 14 Mai 2012**

**13h15 Welcome**

**13h45 Welcome Addresses and Opening Remarks:**

**Jean-François Bach** (Secrétaire perpétuel of the Académie des Sciences)

**Joerg Hacker** (President of the German National Academy of Sciences Leopoldina)

**Dorothee Dzwonnek** (General Secretary of the German Research Foundation)

**David Holden** (Fellow of The Royal Society)

**Pascale Cossart** (Member of the Academie des Sciences)

**14h15 Chair : Pascale Cossart**

### **Proteomics**

**14h30 Ruedi Aebersold** (Institute of Molecular Systems Biology, Switzerland)

Quantitative Analysis of Microbial Proteomes

*14h50 Discussion*

### **Small RNAs and new regulatory mechanisms**

**15h00 Jorg Vogel** (University of Wuerzburg, Germany)

An RNA perspective on bad microbes and their eukaryotic hosts

*15h20 Discussion*

**15h30 Jörgen Johansson** (Umea University, Sweden)

Light and dark oscillations coordinate multicellular differentiation in *Listeria* colonies through a blue-light receptor

*15h50 Discussion*

**16h00-16h30 Coffee break**

**16h30 Rotem Sorek** (Weizmann Institute, Israel)

Genomic probing into the immune system of bacteria

*16h50 Discussion*

**17h00 Steve Busby** (University of Birmingham, UK)

Transcriptional regulation in *E. coli*: simple and complex promoters

*17h20 Discussion*

### **Cellular Microbiology**

**17h30 Hélène Bierre** (Institut Pasteur, France)

Bacterial targeting of chomatin : the *Listeria* paradigm

*17h50 Discussion*

18h30-20h30 **Buffet dinner for all participants**

**Tuesday, May 15<sup>th</sup> 2012 / Mardi 15 Mai 2012**

**Bacterial communities / Signaling**

**8h45 Chair : Joerg Hacker**

**9h00 Jean Marc Ghigo** (Institut Pasteur, France)  
Airborne interactions mediated by volatile molecules produced by bacterial biofilm communities

9h20 *Discussion*

**9h30 Regine Hengge** (Institut für Biologie, Mikrobiologie, Freie Universität Berlin, Germany)

Cyclic-di-GMP signaling and biofilm formation in commensal and pathogenic *Escherichia coli*.

9h50 *Discussion*

**10h00 Sigal Ben Yehuda** (the Hebrew University of Jerusalem, Israel)  
Intercellular Nanotubes Mediate Bacterial Communication

10h20 *Discussion*

**10h30-11h00 Coffee break**

**Microbiota / Commensalism / Ecology**

**11h00 Chair : Philippe Sansonetti**

**11h15 Eric Pamer** (Memorial Sloan-Kettering Cancer Center, USA)  
Antibiotic effects on the intestinal microbiota of mice and humans

11h35 *Discussion*

**11h45 Stanislav Dusko Ehrlich** (INRA, UMR1319, France)  
Human microbiome in chronic disease

12h05 *Discussion*

**12h15 Roberto Kolter** (Harvard Medical School, USA)  
Exploring Bacterial Communication Through Chemical Ecology

12h35 *Discussion*

**12h45-14h00 Lunch + Posters**

**Microbial Cell Biology / Single Cell analysis**

**14h00 Chair : David Holden**

**14h15 Kenn Gerdes** (Newcastle University, UK)  
Bacterial Persistence by Toxin - Antitoxins

14h35 *Discussion*

**14h45 Richard Losick** (Harvard University, USA)  
Life and Death of a Bacterial Community

15h05 *Discussion*

**15h15 Neeraj Dhar (John Mc Kinney)** (Ecole Polytechnique Fédérale de Lausanne, Switzerland)

Individuality of microbial responses to antibiotics

15h35 *Discussion*

**15h45 Miroslav Radman** (University Paris Descartes, France)  
Robust life of *Deinococcus radiodurans*

16h05 *Discussion*

**16h15-16h45 Coffee break**

### **Manipulation of innate and cellular immunity**

**16h45 Chair : Jürgen Heesemann**

**17h00 Geoffrey Smith** (Cambridge University, UK)  
Suppression of innate immunity by vaccinia virus

17h20 *Discussion*

**17h30 Bruno Lemaitre** (Ecole Polytechnique Fédérale de Lausanne, Switzerland)  
The Drosophila gut : a new paradigm for epithelial immune response

17h50 *Discussion*

**18h00 Maria-Carla Saleh** (Institut Pasteur, France)  
Deciphering the viral persistent infection state through RNAi.

18h20 *Discussion*

**18h30 Armelle Phalipon** (Institut Pasteur, France)  
New insights into the manipulation of host adaptive immunity by *Shigella*

18h50 *Discussion*

**Wednesday, May 16<sup>th</sup> 2012 / Mercredi 16 Mai 2012**

### **Genomics / Synthetic biology**

**9h00 Chair : Richard Moxon**

9h15 **Mark Achtman** (University College, Cork, Ireland)  
Insights from genomic comparisons

9h35 *Discussion*

9h45 **Philippe Marlière** (Isthmus SARL, Paris, France)  
Chemically Modified Organisms

10h05 *Discussion*

**10h15-10h45 Coffee break**

**10H45 Keynote lecture**

**Chair : Richard Moxon**

**Venkatraman Ramakrishnan**

(Laboratory of Molecular Biology, Cambridge, UK). Nobel Prize in Chemistry 2009

11h40 *Discussion*

**12H00 End of the meeting**



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# ABSTRACTS BOOK

### Organizing Committee

**Pascale Cossart** and **Philippe Sansonetti**,  
Académie des sciences,  
**Joerg Hacker** and **Juergen Heesemann**,  
German National Academy of Sciences Leopoldina  
**David Holden** and **Richard Moxon**,  
The Royal Society

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## **An RNA perspective on bad microbes and their eukaryotic hosts**

**Jörg Vogel**

Institute for Molecular Infection Biology, University of Würzburg, Germany

This talk will discuss emerging concepts and mechanisms of gene regulation by small noncoding RNAs (sRNAs) in bacteria, for example, programmed target mRNA decay, seed pairing, and 3' adenosine dependence of those sRNAs that act by the common RNA chaperone, Hfq. Furthermore, I will present examples of how RNA deep sequencing technologies have been facilitating new approaches to the global discovery and functional study of diverse functional sRNAs in many bacterial species, and how these can be used for parallel analysis of gene expression programs in a bacterial pathogen and its eukaryotic host during the course of an infection.

### Selected references

Vogel J & Luisi BF (2011) *Hfq and its constellation of RNA*.  
*Nature Reviews Microbiology* 9(8):578-89

Sharma CM et al. (2010) *The primary transcriptome of the major human pathogen Helicobacter pylori*.  
*Nature* 464(7286):250-5

Papenfort K et al. (2010) *Evidence for an autonomous 5' target recognition domain in an Hfq-associated small RNA*.  
*PNAS* 107(47):20435-40

## **Light and dark oscillations coordinate multicellular differentiation in Listeria colonies through a blue-light receptor**

**Jörgen Johansson**

Umea University, Sweden

*Listeria monocytogenes*, a Gram-positive bacterium frequently found in decaying soil, can occasionally cause serious life-threatening infections. Unlike many other bacteria, *Listeria monocytogenes* is only motile at low temperatures (<30°C), due to a complex regulatory system preventing motility at higher temperatures. We show that colonies of *Listeria monocytogenes* undergo ring-formation (opaque and translucent rings) on agar plates in response to oscillating light/dark conditions. The ring formation is strictly dependent on a blue-light receptor, acting through the stress-sigma factor,  $\sigma^s$ . Expression of the blue-light receptor is induced by H<sub>2</sub>O<sub>2</sub> and a strain lacking Lmo0799 display a reduced survival at oxidative stress. A saturated transposon mutant screening identified 65 different genes/operons required for ring-formation, mainly modulating  $\sigma^s$  activity in response to altered Reactive Oxygen Species (ROS) levels. Due to carbon limitation, the bacteria from the inner part of the colony rapidly enter a resting state, preventing translucent rings to form opaque rings when exposed to light. Our results show that night and day cycles coordinate a differentiation of a *Listeria* colony, by a process requiring a blue-light receptor and  $\sigma^s$ .

## **Genomic probing into the immune system of bacteria**

**Rotem Sorek**

Institute of Science, Department of Molecular Genetics, Israel

Bacteria are constantly exposed to phage attacks in the environment. To survive these attacks, bacteria had developed multiple lines of molecular defense mechanisms such as restriction enzymes, abortive infection mechanisms, and the adaptive immune system CRISPR. We have developed high throughput genomic tools to discover and characterize novel bacterial immune systems that protect against phage. These methods, and the nature of the immune systems we detected, will be discussed in the talk.

## **Transcriptional regulation in *E. coli*: Simple and complex promoters**

**Steve Busby**

School of Biosciences, University of Birmingham, UK

The last decade has seen a renaissance in the study of transcriptional regulation in *Escherichia coli* due mainly to the arrival of whole genome sequences and detailed structural information about the multi-subunit RNA polymerase. Until recently, our knowledge was based on case-by-case studies of favorite regulatory regions. However, the application of genomic methodologies, such as transcriptomics, ChIP-on-chip and bioinformatics, has revealed new insights and unexpected complications. Promoters are the main drivers of bacterial gene expression. Some recent results concerning the integration of different signals at complex *Escherichia coli* promoters will be presented.

### Selected references

*Grainger, D, Lee, D & Busby, S (2009) Direct methods for studying transcription regulatory proteins and RNA polymerase in bacteria. Current Opinion in Microbiology 12 531-535*

*Browning, D, Grainger, D & Busby S (2010) Effects of nucleoid-associated proteins on bacterial chromosome structure and gene expression. Current Opinion in Microbiology 13 773-780*

*Rossiter, A, Browning, D, Leyton, D, Johnson, M, Godfrey, R, Wardius, C, Desvaux, M, Cunningham, A, Ruiz-Perez, F, Nataro, J, Busby, S & Henderson, I (2011) Transcription of the plasmid-encoded toxin gene from enteroaggregative *Escherichia coli* is regulated by a novel co-activation mechanism involving CRP and Fis. Molecular Microbiology 81 179-191*

## **Salmonella effector proteins in the regulation of host membrane trafficking**

**Stéphane Méresse**

Centre d'Immunologie de Marseille-Luminy, Parc Scientifique de Luminy, case 906,  
13288 Marseille cedex 9

Salmonella is a bacterial pathogen. Ingested bacteria cross the intestinal epithelial barrier and proliferate within host cells. Intracellular replication takes place in a membrane-bound compartment called the Salmonella-containing vacuole (SCV). Infected cells are characterized by a profound reorganisation of late endocytic compartments. While lysosomal contents (i.e. lysosomal enzymes) tend to disappear from infected cells, lysosomal membrane glycoproteins (i.e. LAMP) accumulate on SCVs and on membrane tubular structures that extend from the SCV. These modifications involve the translocation into the host cell of bacterial effector proteins.

Among these effector proteins, SifA is required for the SCV stability and the formation of membrane tubular structures. Together with its eukaryotic target SKIP, SifA is also necessary for the removal of kinesin-1, which is recruited on the SCV membrane by the PipB2 effector.

We recently showed that the effector protein SopD2 is responsible for the SCV instability that triggers the cytoplasmic release of a *sifA*- bacterial mutant. Membrane tubular structures that extend from the SCV are the hallmark of Salmonella-infected cells, and until recently, these unique structures had not been observed in the absence of SifA. The deletion of *sopD2* in a *sifA*- mutant strain re-established membrane trafficking from the SCV and led to the formation of new membrane tubular structures, the formation of which is dependent on other Salmonella effector(s). Taken together, our data demonstrate that SopD2 inhibits the vesicular transport and the formation of tubules that extend outward from the SCV and thereby contributes to the phenotypes observed in absence of SifA.

The antagonistic roles played by SopD2 and SifA in the membrane dynamics of the vacuole, and the complex actions of SopD2, SifA, PipB2 and other unidentified effector(s) in the biogenesis and maintenance of the Salmonella replicative niche will be discussed.

## **Bacterial targeting of chomatin : the Listeria paradigm**

**Hélène Bierne**

Institut Pasteur, Paris, France

During infection bacterial pathogens subvert a variety of host cell functions to survive in body tissues and control host defenses. There is growing evidence that bacteria modify chromatin in order to reprogram host cell transcription to their benefit. This is particularly well exemplified by *Listeria monocytogenes*, an intracellular bacterium that can control gene expression at the chromatin level in two ways, either indirectly by activating signaling pathways in the cytosol of host cells or directly by manipulating the chromatin-remodeling machinery in the nucleus. In particular, *L. monocytogenes* tightly controls the secretion of a nucleomodulin, LntA, to manipulate the function of BAHD1, the core component of a novel chromatin-silencing complex. Listeria infection of epithelial cells triggers BAHD1-mediated repression of a set of immunity genes. When produced, LntA translocates to the nucleus and counteracts this repression. This positive and negative control of a chromatin repressor allows Listeria to tune type I and type III interferon responses. In this study, we highlight a new strategy used by a bacterial pathogen to exploit nuclear processes and we present an original approach to discover regulators and target genes of chromatin-mediated epigenetic regulations.

## **Airborne interactions mediated by volatile molecules produced by bacterial biofilm communities**

**Jean-Marc Ghigo**

Genetics of Biofilms Laboratory, URA CNRS 2172, Department of Microbiology,  
Institut Pasteur, 25 rue du Docteur Roux, Paris, France

Bacteria produce and sense a high diversity of signals to adapt their behaviors to changing environments. Although volatile compounds produced by fungi and plants were shown to modify bacterial activities, the influence of bacterial volatile molecules on bacteria themselves remained poorly explored. We investigated the role of volatile compounds produced by high-cell density cultures or biofilm communities and characterized several secondary metabolites affecting antibiotic resistance, stress responses and other bacterial phenotypes, in physically separated bacteria. These findings show that airborne chemical interferences between bacteria contribute to bacterial biology and that volatile molecules could constitute ideal infochemicals in heterogeneous environments (soil, gut, etc.) colonized by bacteria.

## **Cyclic-di-GMP signaling and biofilm formation in commensal and pathogenic *Escherichia coli***

**Regine Hengge**

Institut für Biologie – Mikrobiologie, Freie Universität Berlin, 14195 Berlin, Germany

The ubiquitous bacterial signaling molecule cyclic-di-GMP, which is produced by diguanylate cyclases (DGC, carrying GGDEF domains) and degraded by specific phosphodiesterases (PDE, with EAL or HD-GYP domains), regulates transitions between the motile-planktonic and sedentary-multicellular biofilm “life-styles“ (1). c-di-GMP controls a variety of targets, including transcription and the activities of enzymes and complex cellular structures (2). Many bacterial species possess multiple GGDEF/EAL proteins (29 in *E.coli*), which has led to the concept of temporal and functional sequestration of locally acting c-di-GMP control modules (1). Some GGDEF/EAL domain proteins (4 in *E.coli*) have degenerate GGDEF/EAL motifs, are enzymatically inactive and act by direct macromolecular interactions (3).

The presentation will focus on a complete locally acting c-di-GMP control module that consists of a DGC, a PDE, a c-di-GMP-binding transcription factor, which tightly interact in a signaling complex that controls the expression of a key biofilm regulator. Within this complex, the transcription factor binds exclusively to 'internal' c-di-GMP generated by the DGC within the complex. Moreover, this process is allosterically controlled by 'external' c-di-GMP generated by other DGCs, which establishes a regulatory cascade of different c-di-GMP control modules. In addition, data on c-di-GMP signaling and biofilm formation obtained with the recent German EHEC outbreak strain and related *E. coli* strains will be presented.

### Selected references

1. Hengge, R. 2009. Principles of cyclic-di-GMP signaling. *Nature Rev. Microbiol.* 7:263-273.
2. Pesavento, C., G. Becker, N. Sommerfeldt, A. Possling, N. Tschowri, A. Mehrlis, and R. Hengge. 2008. Inverse regulatory coordination of motility and curli-mediated adhesion in *Escherichia coli*. *Genes Dev.* 22:2434-2446.
3. Tschowri, N., S. Busse, and R. Hengge. 2009. The BLUF-EAL protein YcgF acts as a direct anti-repressor in a blue light response of *E.coli*. *Genes Dev.* 23:522-534.

## **Intercellular Nanotubes Mediate Bacterial Communication**

**Sigal Ben-Yehuda**

Department of Microbiology and Molecular Genetics, Institute for Medical Research, Israel-Canada (IMRIC), Faculty of Medicine, POB 12272, The Hebrew University of Jerusalem, 91120, Jerusalem, Israel

Bacteria are known to communicate primarily via secreted extracellular factors. We identified a previously uncharacterized type of bacterial communication mediated by nanotubes that bridge neighbouring cells. Using *B. subtilis* as a model organism we visualized transfer of cytoplasmic fluorescent molecules between adjacent cells. Additionally, by co-culturing strains harbouring different antibiotic resistance genes, we demonstrated that molecular exchange enables cells to transiently acquire non-hereditary resistance. Furthermore, non-conjugative plasmids could be transferred from one cell to another, thereby conferring hereditary features to recipient cells. Electron microscopy revealed the existence of variously sized tubular extensions bridging neighbouring cells, serving as a route for exchange of intracellular molecules. Our recently developed fluorescence microscopy approaches enable us to visualize nanotubes formation in living cells and to follow their activity. Nanotubes also are formed in an interspecies manner, suggesting that they represent a major form of bacterial communication in nature, providing a network for exchange of cellular molecules within and between species.

## **Exploring Bacterial Communication Through Chemical Ecology**

**Roberto Kolter**

Harvard Medical School, Boston, USA

Chemical signaling is basic to all of life, shaping how organisms develop, thrive, and interact with other living systems. Bacteria are ideal for studying such chemical communication systems, since they are both easy to manipulate genetically and prolific producers of compounds. I will focus the presentation on studies of intraspecies and interspecies signaling events that affect the outcome of microbial differentiation.

## **Antibiotic effects on the intestinal microbiota of mice and humans**

**Ying Taur, Joao Xavier, Charlie Buffie, Robert Jenq, Carles Ubeda, Marcel van den Brink and Eric Pamer**

Infectious Diseases Service and Bone Marrow Transplantation Service,  
Department of Medicine, Immunology Program, Computational Biology Program,  
Sloan-Kettering Institute, Lucille Castori Center for Microbes, Inflammation and  
Cancer, Memorial Sloan-Kettering Cancer Center, New York, NY 10065, USA

Infections caused by antibiotic-resistant bacteria generally begin with colonization of mucosal surfaces, in particular the intestinal epithelium. Mammalian gastrointestinal mucosal surfaces are inhabited by thousands of different bacterial species, which, in aggregate, are referred to as the microbiota. The intestinal microbiota provides resistance to infection with highly antibiotic-resistant bacteria by inducing the production of antimicrobial factors in intestinal epithelial cells. Depletion of the microbiota with an antibiotic cocktail containing metronidazole, neomycin and vancomycin (MNV) diminishes the expression of Reg3 $\gamma$ , an antimicrobial factor that kills Gram-positive bacteria, thereby decreasing resistance to intestinal colonization by Vancomycin Resistant Enterococcus (VRE). We have characterized antibiotic-induced changes in the intestinal microbiota and the impact of these changes on susceptibility to intestinal colonization with VRE. Antibiotic treatment decreases the density of intestinal bacteria and dramatically alters the intestinal microbiota. Cessation of antibiotic treatment results in bacterial density increase, however the recovered microbiota has decreased frequencies of bacteria belonging to the Bacteroidetes phylum and the Lactobacillaceae family and increased frequencies of bacteria belonging to the Clostridium and Enterococcus genus and the Enterobacteriaceae family. While mice that have not received antibiotics are resistant to colonization with VRE, antibiotic treatment enables VRE to dominate the intestinal microbiota, reaching frequencies of 95% in the ileum and cecum. Once established, VRE remains a dominant member of the microbiota upon cessation of antibiotic treatment. Characterization of the microbiota colonizing patients undergoing allogeneic hematopoietic stem cell transplantation revealed that antibiotic treatment of humans also enables VRE and bacteria belonging to the Streptococcaceae and Enterobacteriaceae families to dominate the intestinal microbiota. The impact of intestinal domination by different bacterial taxa on clinical outcomes following allogeneic hematopoietic stem cell transplantation is being investigated.

## **Human microbiome in chronic disease**

**Stanislav Dusko Ehrlich**  
INRA, UMR1319, France

## Bacterial Persistence by Toxin – Antitoxins

**Etienne Maisonneuve, Kristoffer Winther and Kenn Gerdes**

Institute for Cell & Molecular Biosciences, Newcastle University, NE2 4AX, UK

The phenomenon “bacterial persistence” was discovered in 1944 by Joseph Bigger working on how penicillin kills *Staphylococcus aureus*. Bigger observed that penicillin did not consistently sterilize cultures of growing bacteria. Bigger called the surviving bacteria for “persisters” and correctly assumed that they were slowly-growing or dormant cells that had become tolerant to the drug. Since then, it has become clear that almost all bacteria form persisters.

*E. coli* K-12 has 11 type II toxin – antitoxin (TA) loci, 10 of which encode RNases that reversibly inhibit translation by degradation of mRNA. The 11th TA locus encodes a Ser/Thr kinase that probably inhibits translation by phosphorylation of EF-Tu. Interestingly, vapBC loci encode RNases that also inhibit translation reversibly but by cleavage of initiator tRNAs<sup>1</sup>. It should be noted that vapBC loci are extremely abundant in the highly persistent human pathogen *M. tuberculosis*<sup>2</sup>.

We showed recently that deletion of the 10 TA loci of *E. coli* K-12 that encode RNases reduces persistence ca. 200-fold<sup>3</sup>. A similar, but much stronger phenomenon was observed with a pathogenic Gram-negative bacterium. The pathway that leads to TA locus activation and how this knowledge may further our understanding of the mechanisms behind the persistence phenomenon will be discussed.

### Selected references

1. Winther, K. S. & Gerdes, K. (2011). Enteric virulence associated protein VapC inhibits translation by cleavage of initiator tRNA. *Proc. Natl. Acad. Sci. U. S. A* 108, 7403-7407.
2. Pandey, D. P. & Gerdes, K. (2005). Toxin - antitoxin loci are highly abundant in free-living but lost from host-associated prokaryotes. *Nucleic Acids Res.* 33, 966-976.
3. Maisonneuve, E., Shakespeare, L. J., Jorgensen, M. G. & Gerdes, K. (2011). Bacterial persistence by RNA endonucleases. *Proc. Natl. Acad. Sci. U. S. A* 108, 13206-13211.

## Life and Death of a Microbial Community

**Rich Losick**

Harvard University, USA

*B. subtilis* forms multicellular communities known as biofilms on solid surfaces, air/liquid interfaces, and in submersion on the roots of plants. Cells in the biofilm are held together by an extracellular matrix consisting of exopolysaccharide and an amyloid-like fiber. Biofilms typically have a finite lifetime, characterized by eventual disassembly. I will discuss the mechanisms that govern biofilm assembly and disassembly. Biofilm formation is triggered by unknown environmental signals that are sensed by the sensor kinases, with one kinase playing a special role in sensing signals released from plant roots. Activation of the kinases sets in motion events that turns on genes for the matrix. Matrix gene expression is governed by a novel double-negative loop involving protein-protein and protein-DNA interactions. The loop behaves like a self-reinforcing switch and when switched on locks cells in the state of matrix production. The same switch also governs stochastic switching between states of chaining and motility during growth. Finally, I will report on two factors that promote exit from the biofilm. These are certain non-canonical D-amino acids and the polyamine norspermidine, which are produced late in the life cycle of the biofilm. D-amino acids are incorporated into the cell wall, causing cells to jettison the amyloid fibers whereas norspermidine interacts specifically with, and collapses, exopolysaccharide polymers.

## **Individuality of microbial responses to antibiotics**

**Neeraj Dhar and John D. McKinney**

Laboratory of Microbiology and Microsystems, Swiss Federal Institute of Technology  
in Lausanne (EPFL), 1015 Lausanne, Switzerland.

Bacterial cells behave as individuals. Mutation and genetic exchange are important drivers of bacterial individuation, but these events are relatively rare. At higher frequencies, genetically identical cells display metastable variation in their growth rate, response kinetics, stress resistance, and other quantitative phenotypes. These cell-to-cell differences arise from cell-intrinsic but non-genetic sources, for example, stochastic fluctuations in gene expression and asymmetric partitioning of components at cell division. Temporal variation at the single-cell level generates phenotypic diversity at the population level. This diversity is critical for bacterial persistence in fluctuating environments because it ensures that some individuals will survive a potentially lethal challenge that would otherwise extinguish the population. A medically relevant example is the refractoriness of bacterial infections to chemotherapy, which has been attributed to spontaneous phenotypic variants ("persisters") that survive despite prolonged antibiotic exposure. The persisters are not antibiotic resistant mutants and it is unclear why they tolerate antibiotics that kill their genetically identical siblings. Our studies focus on the mechanistic basis of the reversible persister switch in bacteria. We use long-term automated timelapse fluorescence microscopy in conjunction with purpose-built microfluidic and microelectromechanical systems to study bacterial responses to antibiotics at the single-cell level. Our observations contradict the conventional notion that persistence is attributable to pre-existing subpopulations of dormant cells. Instead, we find no correlation between the growth rates of individual cells and their probability of survival during antibiotic exposure. In the specific case of isoniazid, a frontline anti-tuberculosis drug, we find that the isoniazid-activating enzyme catalase-peroxidase is expressed in apparently random pulses that are correlated with ensuing cell death. We also find that catalase pulsing and cell fate (death or persistence) are strongly correlated between sibling cells, suggesting a role for metastable non-genetic inheritance in these phenomena. Our observations are consistent with the idea that stochastic fluctuations in gene expression at the single-cell level can specify the fate of individual cells and cell populations in changing and stressful environments.

### **The *Drosophila* gut: a new paradigm for epithelial immune response**

**Bruno Lemaitre**

Global Health Institute, Ecole Polytechnique Fédérale de Lausanne, Station 19, CH-  
1015 Lausanne, Switzerland

The gut combines and integrates very different physiological functions required for maintaining the equilibrium of the whole organism. In addition to its role in digestion, it is the main entry route for pathogens, and a reservoir for resident bacteria that must be tolerated. Finally, the intestinal epithelium undergoes a constant renewal required to maintain the integrity of this barrier. However, little is known about how these functions are regulated and coordinated, or what mechanisms are required to ensure gut homeostasis upon exposure to external challenges such as bacterial infection. Using an integrated approach, we are studying the mechanisms that make the gut an efficient and interactive barrier despite its constant interactions with microbes. We also focus our attention on the regulatory mechanisms that restore gut normal function upon challenge with bacteria. Our projects utilize integrated approaches to dissect not only the gut immune response, but also gut homeostasis and physiology in the presence of microbiota, as well as strategies used by entomopathogens to circumvent these defenses. We believe that the fundamental knowledge generated on *Drosophila* gut immunity will serve as a paradigm of epithelial immune reactivity and have broader impacts on our comprehension of animal defense mechanisms and gut homeostasis.

## Life, death and resurrection - the way of the *Deinococcus radiodurans*

**Miroslav Radman\*\* (speaker)**

Anita Krisko° and Svjatlana Cvjetan° (coauthors)

\*Inserm U1001, Faculté de Médecine, Université R. Descartes, 156, rue de Vaugirard, 75015- Paris, France. °Mediterranean Institute for Life Sciences, 21000 Split, Croatia.

*Deinococcus radiodurans* survives long-term abiosis in deserts and in atmosphere/stratosphere exposed, in a desiccated state, to excessive UV radiation<sup>1</sup>. The comparative molecular biology of *D. radiodurans*<sup>1,2</sup>, robust animals<sup>3</sup> and standard species teaches that the prime target in cell death is function (proteome) rather than information (genome) because proteins repair DNA and not vice versa. Extreme biological robustness is accounted for by a constitutive small molecular weight protection against protein (not DNA) damage<sup>2,4</sup> whereas proteins of standard and robust species have evolved largely oxidation-resistant conformations. Exceptional DNA repair in *D. radiodurans*<sup>5</sup> is due to the protection of a standard repair system from oxidative damage<sup>6</sup>. We followed the fate of radiation-damaged DNA and proteins and identified the time of decision between life and death. The point of no-return in cell death by radiation, in *Escherichia coli* and *Deinococcus radiodurans*, is cell membrane depolarization and permeability - caused by delayed synthesis of oxidized aberrant membrane proteins - resulting in the exit of small metabolites required for vital biosynthetic processes. Natural or artificial prevention of protein oxidation prevents the loss of cellular fitness and cell death. If this cell death paradigm holds for human cells, the strategies of cancer therapy should be revisited.

### Selected references

1. Slade, D. & Radman, M; "Oxidative Stress Resistance in *Deinococcus radiodurans*" *Microbiol. Mol. Biol. Reviews* (2011) 75: 133-191.
2. Krisko, A. & Radman, M. "Protein damage and death by radiation in *Escherichia coli* and *Deinococcus radiodurans*" *Proc. Natl. Acad. Sci. USA* (2010) 107: 14373-14377.
3. Krisko, A., Leroy, M., Radman, M. & Meselson, M. "Extreme anti-oxidant protection against ionizing radiation in bdelloid rotifers" *Proc. Natl. Acad. Sci. USA* (2012) 109 : 2354-7.
4. Daly MJ, Gaidamakova EK, Matrosova VY, et al. Small-molecule antioxidant proteome-shields in *Deinococcus radiodurans*. *PLoS One* (2010) 5:e12570.
5. Zahradka, K., Slade, D., Bailone, A., Sommer, S., Averbek, D., Petranovic, M., Lindner, A.B. & Radman, M. Reassembly of shattered chromosomes in *Deinococcus radiodurans*. *Nature*, (2006) 443: 569-573.
6. Slade, D, Lindner, AB, Paul, A, & Radman, M. "Recombination and Replication in DNA Repair of Heavily Irradiated *Deinococcus radiodurans*" *Cell*, (2009) 136: 1044-1055.

## **Suppression of innate immunity and rapid spreading by vaccinia virus**

**Geoffrey L Smith**

Department of Pathology, University of Cambridge, Tennis Court Road,  
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The mammalian host provides a hostile environment for viruses and to replicate and be transmitted efficiently to new hosts viruses must spread rapidly and evade the host innate response to infection. To study how viruses do this we have utilised vaccinia virus, a large DNA virus that replicates in the cell cytoplasm and is famous as the live vaccine to eradicate smallpox.

By measuring the rate of spread of vaccinia virus across a lawn of susceptible cells, it was evident that the virus was spreading across each cell in 1.2 h and this was about 4-5-fold faster than could be explained by the replication kinetics of the virus (5-6 h to produce the very first new virions). Therefore, virus spread could not be explained by an iterative process of infection, replication and release and there must be another new mechanism. Investigation showed that shortly after a cell was infected vaccinia virus expressed a complex of 2 proteins on the cell surface that caused virions trying to re-infect the same cell to be repelled on actin projections. These virions would continue to be repelled until they reached other cells that were uninfected. Thus the virus could spread rapidly without the need to replicate in every cell along the way.

But rapid spread is not sufficient unless the virus can combat the innate immune system, which would otherwise suppress virus replication. Consequently, vaccinia virus expresses a plethora of proteins that can act either within or outside an infected cell to block the innate response to infection. To illustrate this, a new pathway by which the innate immune system senses and responds to foreign DNA, and how vaccinia virus blocks this, will be described.

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## **Deciphering the viral persistent infection state through RNAi.**

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Host-pathogen interactions are complex and dynamic processes. The outcome of such interactions is determined by the ability of the pathogen to circumvent the host immune system and the capacity of the host to respond. Such an interaction may result in the elimination of the pathogen, in asymptomatic carriage, or in mild to serious disease.

Insect-virus interactions are interesting models to understand the complexity of this process, since many insects act as vectors for an increasing number of emerging human viral diseases. In this case, the viruses are exposed to different antiviral immune responses of the mammalian and the insect hosts. A mastery of the insect immune system could lead to a better control over the transmission of disease. In fact, many insect viruses develop a persistent infection during which cells are infected and produce viral particles, yet without clear signs of infection. In this context a key question is how viruses establish a persistent infection in insects.

To address this question we use as a model the drosophila S2 cell line persistently infected (S2p) with Flock House virus (FHV). This virus, as many other insect viruses, triggers an effective RNA interference (RNAi) response in *D. melanogaster* that is essential to survive viral infection. Interestingly, S2p cells show no obvious signs of infection but produce viable viral particles, which retain their virulence in S2 naive cells as well as in adult flies. Altogether, these data suggest that the persistent infection state is mainly controlled by the insect antiviral response, RNAi. To gain insight into the mechanism underlying the switch from acute to persistent infection, we analyzed the RNAi-mediated antiviral response in both conditions by deep sequencing. These and other efforts to understand how the host-pathogen interaction evolves under RNAi pressure will be discussed.

## **New insights into the manipulation of host adaptive immunity by Shigella**

**Armelle Phalipon**

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*Shigella* is an enteroinvasive pathovar of *Escherichia coli* that causes shigellosis or bacillary dysentery, an acute recto-colitis characterized by a rapid influx of polymorphonuclear neutrophils (PMNs) to the lamina propria, leading to massive tissue destruction. *Shigella*'s pathogenesis relies on the expression of a type III secretion system (T3SS) and its secreted effector proteins. Although natural infection elicits protection against subsequent infections, several infection episodes are required to generate protective immunity, and memory is of short duration. The strategy employed by *Shigella* to dampen the acquired immune defense system is still ill defined. Our recent work has been focused on *Shigella*-T lymphocyte cross-talks. We have shown that the *Shigella*-induced pro-inflammatory environment is responsible for the predominant priming of *Shigella*-specific CD4<sup>+</sup> Th17 cells (Sellge et al., 2010). *Shigella*-specific CD8<sup>+</sup> T cells are not primed upon infection, which is striking given the intracellular lifestyle of this bacterium (Jehl et al., 2011; Sellge et al., 2010). Interestingly, *Shigella* has also evolved strategies to subvert the host acquired immunity by directly targeting lymphocytes. We recently demonstrated in vitro that activated, as opposed to unactivated, human CD4<sup>+</sup> T cells are susceptible to *Shigella* invasion, and that invasion impairs their migration towards a chemoattractant stimulus. In the absence of invasion, injection of T3SS effectors upon *Shigella*-T cell contact is sufficient to alter T cell migration (Konradt et al., 2011). By assessing T cell targeting in vivo, we demonstrated the dramatic impact of *Shigella* on the CD4<sup>+</sup> T cell dynamics (Salgado-Pabon et al. submitted).

# Insights from genomic comparisons of genetically monomorphic bacterial pathogens

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Some of the most deadly bacterial diseases, including leprosy, anthrax and plague, are caused by bacterial lineages with extremely low levels of genetic diversity, so-called “genetically monomorphic bacteria”. It has only become possible to analyze the population genetic analysis of such bacteria since the recent advent of high-throughput comparative genomics. The genomes of genetically monomorphic lineages contain very few polymorphic sites, which often reflect unambiguous clonal genealogies. Some genetically monomorphic lineages have evolved in the last decades, e.g. antibiotic resistant *Staphylococcus aureus*, whereas others have evolved over several millennia, e.g. the cause of plague, *Yersinia pestis*. Based on recent results, it is now possible to reconstruct the sources and the history of pandemic waves of plague by a combined analysis of phylogeographic signals in *Y. pestis* plus polymorphisms found in ancient DNA. Different from historical accounts based exclusively on human disease, *Y. pestis* evolved in China, or the vicinity, and has spread globally on multiple occasions. These routes of transmission can be reconstructed from the genealogy, most precisely for the most recent pandemic that was spread from Hong Kong in multiple independent waves in 1894.

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## **Chemically Modified Organisms**

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# Quantitative Analysis of Microbial Proteomes

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The value of quantitative measurements in proteomics has long been recognized for microbiology and life science research in general. Commonly, in such studies the proteins contained in two or a few samples are identified and their relative abundances are determined.

In this presentation we will discuss recent technical advances that move the field beyond comparative proteomics. The first advance is the use of selected reaction monitoring mass spectrometry to accurately quantify any protein of a proteome (1). The second is the development of quantitative methods for the determination of the cellular concentration of proteins in microbial cells (2, 3, 4) at high throughput, and third, is new data acquisition and analysis method –SWATH-MS– that generates near complete, permanent digital maps of microbial proteomes that can be repeatedly and perpetually re examined in silico (5). Collectively, these techniques represent an extension of the quantitative proteomics toolbox that is available to researchers studying microbial proteomes.

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## Two mechanisms for quality control of translation in bacteria

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In response to stress, bacteria have enzymes that cleave mRNA in the ribosome to shut down translation. Ribosomes stalled on prematurely truncated or defective messages are rescued by tmRNA, a molecule that acts as both a tRNA and an mRNA. This talk will describe work on the ribosomal endonuclease RelE that cleaves messages inside ribosomes and also how tmRNA in conjunction with a partner protein SmpB can facilitate decoding for defective messages even in the absence of a codon.