Pascale Cossart

Molecular and Cellular Basis of Infectious Diseases: the *Listeria* Paradigm

Thank you very much for this presentation. It is really a huge honour to receive this Prize. I will convey my thanks appropriately tomorrow, but today I want to say that it was a joy to receive the phone call that announced my selection for the award of the 2013 Balzan Prize.

Infectious diseases and in particular bacterial infections are a leading cause of mortality worldwide. They are responsible for 15,000,000 deaths per year – about a quarter of annual deaths worldwide, in particular young children in underdeveloped countries. It is also important to recall that certain cancers are associated with an infectious agent. 15-20% of cancers are caused by either a virus or bacteria. The reappearance of old pathogens, the emergence of new pathogens, the increasing number of antibiotic resistant strains and bioterrorism threats are stressing the urgent need for new antibiotics or new therapeutic strategies. We believe that a prerequisite for the design and generation of novel anti-infectious drugs is a detailed knowledge of infectious processes; it is in this framework that our group is analyzing the infectious process induced by the food-borne human pathogen *Listeria monocytogenes*.

In 1986, we chose to work on this facultative intracellular bacterium primarily because bacterial diseases due to intracellular pathogens were increasingly responsible for important public health problems, and because *L. monocytogenes*, owing to a number of specific properties, appeared as a model system to study intracellular parasitism. It also has the unique property of being able during infection to breach three body-barriers, the intestinal, the placental and blood-brain barriers.

In 2013, this bacterium ranks among the best documented intracellular pathogens. Our group has largely contributed to the characterization of the major virulence factors, to the identification of their cellular ligands, to the discovery of the complex signaling pathways that derive from their interactions, to our knowledge of the cell biology of the bacterial entry process and the cell-to-cell spread, of the resistance of the bacterium to host cell defence strategies and to the relevance of some of these events *in vivo*, in particular in the crossing of two host barriers, the intestinal and the placental barriers.

By studying *Listeria* and listeriosis our group has established several new concepts in different areas of biology, i.e., infection biology, cell biology, and fundamental microbiology.

For the last four years, our five main research themes and objectives have been:

1. Identification of new bacterial components involved in virulence
   A. Role of non-coding RNAs in virulence and new RNA-mediated regulatory mechanisms in bacteria
   B. Characterization of new virulence factors

2. The unexplored facets of the cell biology of infections
   A. New endocytic and cytoskeletal components involved in bacterial entry into cells and cell-to-cell spread
   B. Post translational modifications during infection
   C. The role of mitochondria and mitochondrial dynamics during infection

3. The manipulation of host genetic information by bacteria
   A. Histone modifications upon Listeria infection
   B. Hijacking the heterochromatinisation machinery

4. Unexplored aspects of the intestinal phase of the infection by *Listeria*
   A. Role of commensals
   B. Role of NK cells (collaboration with Eric Vivier, CIML Marseille)

5. Crossing of host barriers by *Listeria* (collaboration with Marc Lecuit, IP)

Scientific activity

Our scientific activity during 2008-2013 has essentially led to:

Objective 1

1. The description of the transcriptional landscape of *Listeria* during the switch from saprophytism to virulence
2. The discovery of novel types of RNA-mediated regulation in bacteria
3. The identification of new virulence factors

Objective 2

4. The establishment of a new role for clathrin in the reorganization of the actin cytoskeleton during bacterial entry and adhesion
5. The discovery of a lipid phosphatase controlling the entry of *Listeria* into cells
6. The elucidation of a role of septins in entrapping intracytosolic bacteria targeted to autophagy
7. The first report on a role for SUMOylation in bacterial infection

8. The demonstration of the key role of mitochondrial dynamics during infection and of an atypical mitochondrial fission mechanism

Objective 3

9. The discovery that dephosphorylation of Serine10 of histone H3 is mediated by K+ efflux via LLO
10. The identification of the role of SIRT2 in the deacetylation of histone H3 and in infection
11. The characterization of BAHD1 as a new heterochromatinization factor
12. The demonstration that *Listeria* remodels chromatin during infection via an LntA/BAHD1 interaction
13. The discovery that *Listeria* induces the production of interferon type III during infection

Objective 4

14. The demonstration of the impact of lactobacilli on oral infection by *Listeria*
15. The identification of an NK cells subset critical for *Listeria* infections (coll E. Vivier)

Objective 5

16. The elucidation of a transcytosis phenomenon in globlet cells during infection *in vivo* (coll M. Lecuit)

Key papers are cited in the paragraphs below and references given at the end.

The transcriptional landscape of L. monocytogenes *during the switch from saprophytism to virulence*

The transcription of the entire *L. monocytogenes* genome was examined by the use of tiling arrays and RNAs from wild type and mutant bacteria grown *in vitro*, *ex vivo* and *in vivo* (Toledo-Arana et al. 2009). This led to the establishment of the first complete bacterial operon map and the discovery of far more diverse types of RNAs than expected: in addition to 50 small RNAs (smaller than 500 nucleotides), at least two of which are involved in virulence in mice, this study led to the identification of long antisense RNAs covering several open reading frames and long overlapping 5' and 3' untranslated regions. It also showed that riboswitches can act as terminators for upstream genes and that when *Listeria* reaches the host intestinal lumen, an extensive

transcriptional reshaping occurs with a SigB-mediated activation of virulence genes. In contrast, in blood, the pleiotropic regulator PrfA controls transcription of virulence genes. Remarkably, several non-coding RNAs absent in the non-pathogenic species *Listeria innocua* exhibit the same expression patterns as the virulence genes. Together these data have unraveled successive and coordinated global transcriptional changes during infection and pointed to previously unknown regulatory mechanisms in bacteria.

We next used RNA-Seq in collaboration with the laboratory of R. Sorek at the Weizmann Institute and identified all the start sites as well as the processing sites which function during transcription (Wurtzel et al. 2012). This study, which was performed after growth of the bacteria in a variety of conditions, showed that the genome encodes for at least 134 non-coding RNAs whose size varies from 50 to 600 nucleotides and 86 antisense RNAs whose sizes vary between 150 and 6500 nucleotides. All our results of RNA-Seq as well as the tiling arrays results together with results of two other groups are available on a public browser: http://weizmann.ac.il/molgen/Sorek/listeria_browser/.

The discovery of novel types of RNA-mediated regulation in bacteria

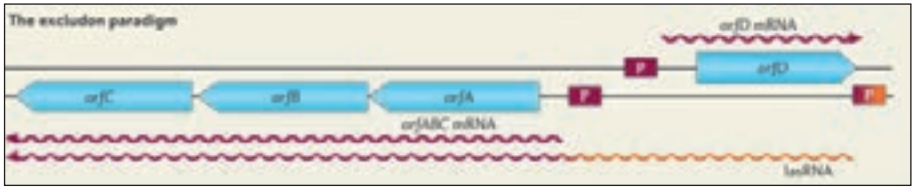
- **Discovery that a riboswitch can generate a regulatory small RNA**

Riboswitches are classically described as ligand-binding elements located in 5' untranslated regions of messenger RNAs, which regulate expression of downstream genes. Upon binding of their ligand, they may adopt a secondary structure, which leads to a premature termination of transcription. The fate of the resulting small transcript had remained elusive. A combination of approaches has led to the discovery that an S-adenosyl methionine (SAM) riboswitch in *Listeria* can generate, in the presence of a high concentration of methionine, a small transcript (SreA), which can hybridize to the 5'UTR of the PrfA message, and inactivate its translation, establishing a link between nutrient availability and virulence gene expression. Together, these results have uncovered a novel role for riboswitches and a new class of regulatory non-coding RNAs in bacteria (Loh et al. 2009).

- **Discovery of a novel type of antisense-mediated regulation in bacteria: the excludon**

Analysis of the antisense RNAs encoded in the *Listeria* genome showed that they can be classified in two classes: the short ones and the very long ones. This led to the identification of a new type of regulation by a long RNA, which acts as both an anti-

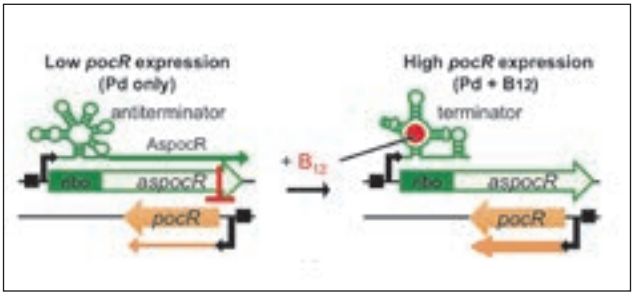
sense RNA and an mRNA. We called the locus encoding such an RNA an excludon. Excludons generally regulate divergent genes involved in similar or mutually exclusive functions (Sesto et al. 2013).



The excludon paradigm. A general representation of an excludon locus, which consists of divergently oriented genes overlapped by a long antisense RNA (lasRNA), is shown. The overlapping lasRNA can act as a negative regulator for genes encoded on the opposite strand, but it can also be used as an mRNA for the genes encoded on the same strand.

- **Discovery of a riboswitch-regulated non-coding RNA**

Riboswitches are, as mentioned above, located in 5' untranslated regions of messenger RNAs. In *L. monocytogenes*, a vitamin B12-binding (B12) riboswitch was identified, not upstream of a gene but downstream, and antisense to the adjacent gene, *pocR*, suggesting it might regulate *pocR* in a nonclassical manner. *PocR* in *Salmonella* regulates propanediol catabolism and B12 biosynthesis, and confers an advantage over commensal bacteria which are unable to use propanediol. We demonstrated that the B12 riboswitch is transcribed as part of a non-coding antisense RNA named AspocR. In the presence of B12, the riboswitch induces transcriptional termination, causing AspocR to be transcribed as a short transcript. In contrast, in the absence of B12, AspocR is transcribed as a long antisense RNA, which inhibits *pocR* expression. Regulation by AspocR ensures that *pocR*, and consequently the *pdu* genes, which are under its control and are involved in propanediol catabolism, are maximally expressed only when B12 is present. Together, this study demonstrates how *pocR* and the *pdu* genes can be regulated by B12 in bacteria, and extends the classical definition of riboswitches from elements governing solely the expression of mRNAs to a wider role in controlling the transcription of non-coding RNAs (Mellin et al. 2013).



Expression of *pocR* is regulated by B_{12} via AspocR. Model showing proposed regulation of *pocR* by AspocR (Pd: propanediol).

The identification of new virulence factors

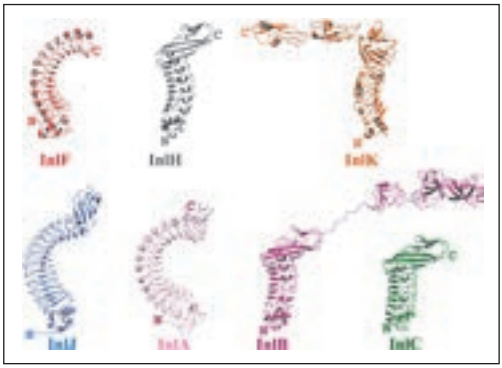
In *Listeria*, the two main invasion proteins internalin (InlA) and InlB are the founding members of the internalin family, which is characterized by an N-terminal domain made of leucine rich repeats. We have characterized several other internalins that are only present in the pathogenic species *L. monocytogenes* and absent in the non-pathogenic *L. innocua*:

- InlC (Gouin et al. 2010). InlC, in contrast to internalin (InlA) and InlB which are attached to the bacterial surface, is secreted in the culture supernatant or in the cytosol of infected cells. InlC interacts with IKK-alpha and inhibits the NF-kappa B pathway by slowing down the phosphorylation and consequently the degradation of the I-kappa B protein. InlC is the first reported *Listeria* protein which interferes specifically with the NF-kappa B pathway and thus downregulates the host response to infection by preventing the production of proinflammatory cytokines and the recruitment of neutrophils at the site of infection.

- InlH (Personnic et al. 2010). InlH is a stress-induced surface protein. It does not contribute to bacterial invasion of cultured cells *in vitro* or of intestinal cells *in vivo*. Strikingly, the reduced virulence of InlH-deficient *L. monocytogenes* strains is accompanied by enhanced production of interleukin-6 (IL-6) in infected tissues during the systemic phase of murine listeriosis, but not by enhanced production of any other inflammatory cytokine tested. Since InlH does not modulate IL-6 secretion in macrophages at least *in vitro*, it may play a role in other immune cells or contribute to a pathway that modulates survival or activation of IL-6-secreting cells. These results strongly suggest that InlH facilitates pathogen survival in tissues by dampening the

inflammatory response. Understanding how it mediates this IL-6 regulation is critical for understanding its role in infection.

- InlK (Dortet et al. 2011; Neves et al. 2013). This protein is not expressed in classic broth media but is expressed in infected animals. As first shown by a two hybrid screen, InlK interacts with MVP, an abundant cytoplasmic ribonucleoprotein, so far poorly characterized, but which is known to interact with microRNAs. This interaction takes place when bacteria get out of the internalization vacuole, and protects bacteria from autophagic protein recruitment. InlK is thus, after ActA, the second protein which mediates bacterial protection against autophagy.



Internalin structures. The structure of LRR repeats of different proteins of the internalin family as determined by crystallography.

We also characterized several non-internalin virulence factors:

- OatA (Aubry et al. 2011). OatA is an O acetyl transferase which modifies the peptidoglycan on the N-acetyl muramic acid residues and, as shown by deleting the gene encoding, this enzyme is critical for virulence. The mutant is more sensitive to lysozyme and to some antimicrobial peptides than the wild type. The analysis of cytokines produced by the mutant revealed that these cytokines are different from those produced by a pgdA mutant, a mutant affecting another peptidoglycan modification and which is also strongly attenuated. OatA is thus a novel virulence factor. These results reinforce the notion that peptidoglycan modifications play a key role in the down-modulation of the immune response.

- LntA (Lebreton et al. 2011) is a small secreted and basic protein which is produced at low levels by bacteria *in vitro*. It is present in *L. monocytogenes* but absent

in other *Listeria* species. LntA targets the nucleus of infected cells, where it interacts with the nuclear protein BAHD1 and inhibits its function, which is to induce heterochromatin formation and gene silencing. Expression of LntA desequesters BAHD1 and triggers expression of interferon stimulated genes (ISGs) during infection. LntA is thus a nucleomodulin which remodels the chromatin during infection, an event critical for infection, as the LntA mutant is attenuated in the murine model.

- LipA (Kastner et al. 2011) is a secreted protein with a conserved motif of conventional tyrosine phosphatases. It is present in other *Listeria* species. We have shown that LipA is secreted by *Listeria* and displays tyrosine as well as lipid phosphatase activity *in vitro* acting on PI(3)P, PI(5)P and PI(3,5)P_2. This dual activity as both a phospho-amino acid and phospholipid phosphatase is in line with the high similarity between the predicted LipA structure and that of phosphatase MPtpB, a virulence factor required for intracellular growth of *Mycobacterium tuberculosis*.

The establishment of a new role for clathrin in the reorganization of the actin cytoskeleton during bacterial entry and adhesion

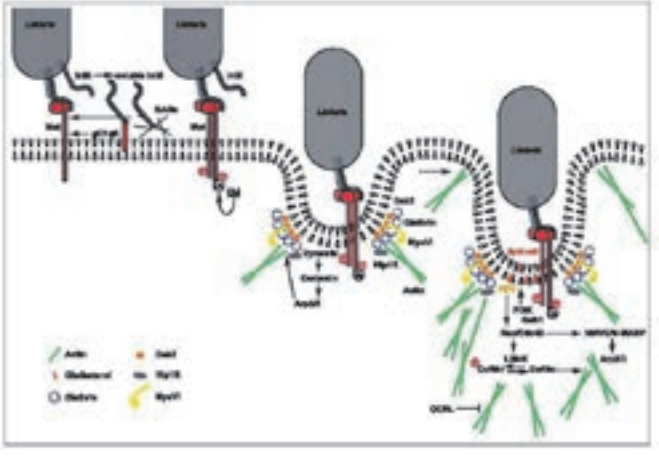
Clathrin is a protein which was classically considered as involved in the internalization of macromolecules or small particules. Our previous results demonstrated its role in bacterial internalization. Phosphorylation of its heavy chain by Src family kinases was shown to be a critical event for both bacterial internalization and adhesion as exemplified by its role in entry of *Listeria* into cells and in adhesion of enteropathogenic *E. coli* to mammalian cells. In addition, the proteins Dab2, and Hip1R were shown to mediate the link between clathrin and the actin cytoskeleton, reinforcing the previous hypothesis and definitively establishing that clathrin acts as a hub for the actin cytoskeletal rearrangements necessary for entry and adhesion (Bonazzi et al. 2011b).

Strikingly, the same machinery is involved in the formation of adherens junctions between epithelial cells. These results established definitively that clathrin is not solely involved in endocytosis, but must also be considered as a molecule involved in actin rearrangements (Bonazzi et al. 2012).

The discovery of a lipid phosphatase controlling entry of **Listeria** *into cells*

L. monocytogenes induces its own entry into a broad range of mammalian cells through interaction of the surface protein InlB with the cellular receptor Met, promoting an actin polymerization/depolymerization process that leads to pathogen engulf-

ment. PI(4,5)P(2) and PI(3,4,5)P(3) are two major phosphoinositides that function as molecular scaffolds, recruiting cellular effectors that regulate actin dynamics during *L. monocytogenes* infection. Because the phosphatidylinositol 5'-phosphatase OCRL dephosphorylates PI(4,5)P(2) and PI(3,4,5)P(3), we investigated whether this phosphatase modulates cell invasion by *L. monocytogenes*. Our observations suggested that OCRL promotes actin depolymerization during infection and that through its phosphatase activity, it restricts *L. monocytogenes* invasion by modulating actin dynamics at internalization sites (Kuhbacher et al. 2012).



Model of InlB mediated entry into non phagocytic cells.

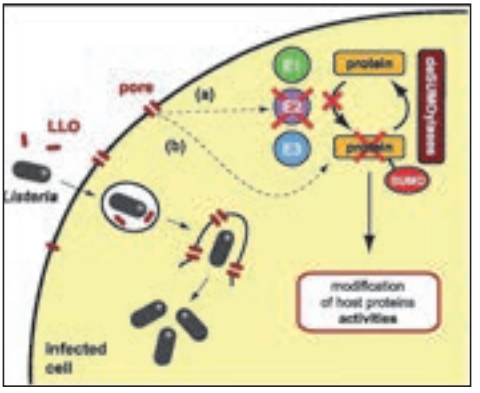
The elucidation of a role for septins in entrapping intracytosolic bacteria targeted to autophagy

Septins are cytoskeletal elements less characterized than actin and tubulin or intermediate filaments. They constitute a large family of GTPases which form non-polar filaments.They play different roles at entry into cells. Interestingly, they are recruited by cytosolic bacteria which polymerize actin and surround the bacteria and the actin comet tails. This occurs for *Listeria* and *Shigella*. More surprising is the fact that septins in the case of *Shigella* can entrap bacteria which have started to polymerize actin and thus prevent the motility of bacteria and the cell-to-cell spread. This phenomenon is amplified by TNF-alpha treatment, a multifactorial cytokine which in particular has

important effects on the cytoskeleton. Bacteria entrapped in the septin cages are targeted to autophagy. Entrapment in septin cages and autophagy are interconnected, as inhibition of autophagy prevents septin caging and inhibition of septin prevents autophagy. This study was the first to report a cellular process counteracting actin-based motility (Mostowy et al. 2010).

The first report on a role for SUMOylation in bacterial infection

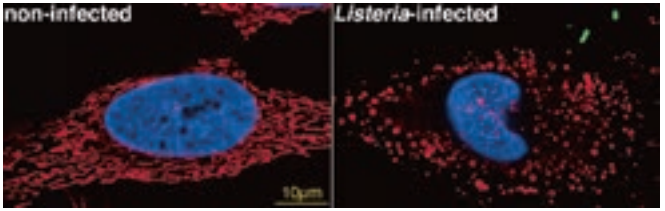
SUMO is a post-translational modification corresponding to the addition of a small ubiquitin-like polypeptide which plays a fundamental role in the cell by controling transcription, genome integrity, intracellular transport, stress responses and other key biological processes. We showed that *Listeria* induces a global deSUMOylation of host proteins through the degradation of Ubc9, the unique enzyme of the SUMO conjugation pathway (Ribet et al. 2010). This degradation is induced by the secreted factor listeriolysin O. The use of inhibitors has revealed that an aspartyl protease is involved in this process. An hyperSUMOylation of cellular proteins impairs infection, highlighting the key role of some SUMOylated proteins in controlling infection. Current investigation aims at identifying the aspartyl protease(s) involved and the SUMOylated proteins involved in infection.



Impairment of host cell SUMOylation during *Listeria* infection. Pore formation by LLO during infection triggers the degradation of the host E2 SUMO enzyme (Ubc9). This leads to a blockade of the SUMOylation machinery and to a global deSUMOylation of host proteins (a). LLO can also trigger the degradation of some SUMOylated proteins (b). This decrease in SUMOylation leads to a modification of host protein activities and is critical for infection.

The demonstration of the key role of mitochondrial dynamics during infection and of an atypical mitochondrial fission mechanism

Mitochondrial dynamics is linked to mitochondrial function in mammalian cells. Video microscopy of mitochondria of *Listeria*-infected cells compared to those in non-infected cells led to the striking observation that bacterial infection leads to a rapid mitochondrial fragmentation, which is specific to *L. monocytogenes* as it is not observed in the case of infection with other invasive enteropathogens. Infection with various *Listeria* mutants allowed us to show that the pore-forming toxin listeriolysin O is the critical factor that induces this fragmentation, and that this protein mediates a calcium influx, which is required for the fission event (Stavru et al. 2011). Fission is transient. It corrolates with a transient shut off in mitochondrial respiration and a drop in ATP. However, mitochondria recover their function, demonstrating that the shut-off is reversible. RNAi depletion experiments of either mitofusins – leading to pre-fragmented mitochondria – or Drp1 – leading to hyperfused mitochondria – allowed us to show that mitochondrial dynamics is critical for efficient infection. Our recent results show that mitochondrial fragmentation is atypical, as it is dynamin-like protein 1 (Drp1)-independent, but is dependent on the endoplasmic reticulum as in the canonical Drp1-dependent fission mechanisms induced, for example, by uncouplers (Stavru et al. 2013).



***Listeria* transiently induces fragmentation of mitochondria**. HeLa cells were infected or not with *Listeria monocytogenes* (green) and labeled for mitochondria (red) and DNA (blue).

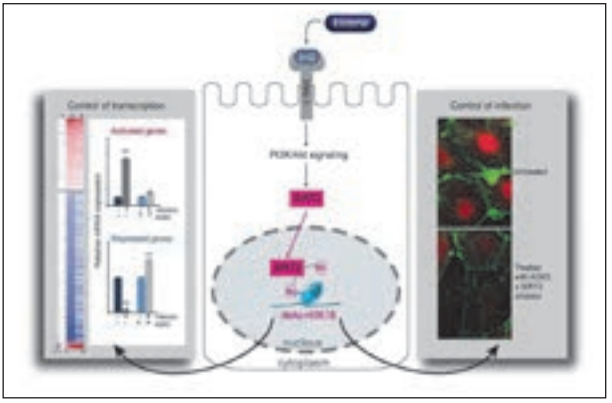
The discovery that dephosphorylation at Serine10 of histone H3 is mediated by K+ efflux via LLO

Following our previous discovery that *L. monocytogenes* dephosphorylates histone H3 through the action of listeriolysin O (LLO), we then showed that an unrelated pore-forming toxin, aerolysin, also provokes H3 dephosphorylation (dePH3). As reported for aerolysin, we showed that LLO also induces a pore-dependent K(+) efflux,

and that this efflux is the signal required for dePH3. In addition, LLO-induced K(+) efflux activates caspase-1. However, we demonstrated that dePH3 is unlinked to this activation. Therefore, our study unveiled K(+) efflux as an important signal leading to two independent events critical for infection, inflammasome activation and histone modification (Hamon and Cossart 2011).

The identification of the role of SIRT2 in the deacetylation of histone H3 and in infection

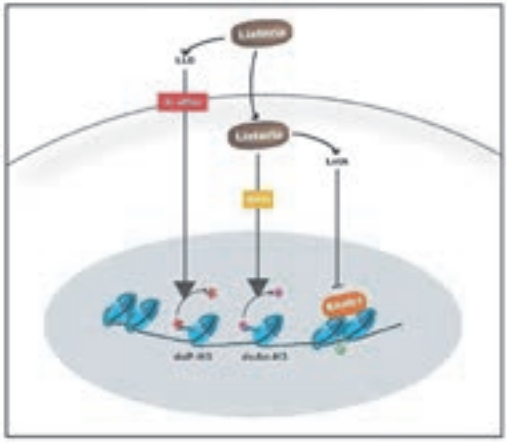
We found that during infection with *L. monocytogenes*, the host deacetylase sirtuin 2 (SIRT2) translocates to the nucleus in a manner dependent on the bacterial factor InlB. SIRT2 associates with the transcription start sites of a subset of genes repressed during infection and deacetylates histone H3 on lysine 18 (H3K18). Infecting cells in which SIRT2 activity was blocked or using SIRT2(-/-) mice resulted in a significant impairment of bacterial infection. Thus, SIRT2-mediated H3K18 deacetylation plays a critical role during infection, which reveals a hitherto undescribed role for SIRT2 and an epigenetic mechanism imposed by a pathogenic bacterium to reprogram its host (Eskandarian et al. 2013).



Model of *Listeria*-induced deacetylation histone H3. *Listeria* induces SIRT2 relocalization from cytoplasm to chromatin where SIRT2 deacetylates H3K18. The consequences of this cascade are the control of host transcription, as illustrated by representative genes regulated by SIRT2, and the control of infection, as assessed by staining cells for the secreted bacterial factor InlC (red), which is overexpressed in the cytosol, and host actin, which is polymerized into comet tails by bacteria (green).

The characterization of BAHD1 as a new heterochromatinization factor

By screening for a protein interacting with LntA, we found a protein which had never been studied. It displayed a BAH domain, a domain known to be present in chromatin associated proteins. By a two-hybrid screen, we identified a series of BAHD1 interactors known to be associated with heterochromatin, e.g., HP1, MBD1 etc. Localisation studies and functional experiments demonstrated that BAHD1 is part of a heterochromatinisation complex which induces gene silencing (Bierne et al. 2009). BAHD1 is a silencer whose identification was permitted through the study of a bacterial factor, reinforcing the view that bacterial pathogens may help to unravel unexpected aspects of cell physiology.

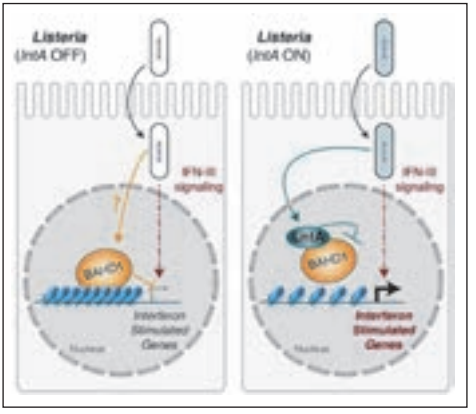


***Listeria* induced chromatin modifications during infection.**

The demonstration that **Listeria** *remodels chromatin during infection via an LntA/ BAHD1 interaction*

LntA is expressed *in vivo* but poorly expressed *in vitro*. In infected tissue cultured cells, constitutively expressed LntA targets the cell nucleus, where it interacts with BAHD1 and inhibits its function, i.e., heterochromatin formation and gene silencing, i.e., LntA desequesters BAHD1 and triggers expression of interferon-stimulated genes (ISGs) during infection. Indeed, interferon type III (IFN-lambda) was shown for the first time to be produced in epithelial cells, upon *Listeria* infection, but ISGs were

produced if and only if LntA was expressed. LntA is thus a nucleomodulin which remodels chromatin during infection, and stimulates ISG expression, an event critical for infection as an LntA mutant is attenuated in the murine model. As a mutant expressing constitutively LntA is also attenuated, our results indicate that the finely tuned expression of LntA *in vivo* is critical for a successful infection (Lebreton et al. 2011). It will be of the highest interest to understand when and where LntA is produced during infection and how the gene LntA is regulated.



Model for LntA/BAHD1-mediated regulation of ISGs.

The discovery that Listeria *induces the production of interferon type III during infection*

Having shown that IFN-lambda, an interferon known to be involved in viral infections, is produced during the infection of epithelial cells with *Listeria,* we analyzed this unexpected expression in detail. In intestinal cells, induction of IFN-lambda genes by *L. monocytogenes* requires bacterial entry and increases further during the bacterial intracellular phase of infection. Other Gram-positive bacteria, *Staphylococcus aureus, Staphylococcus epidermidis* and *Enterococcus faecalis*, also induced IFN-lambda genes when internalized by intestinal cells. In contrast, Gram-negative bacteria *Salmonella enterica* serovar *Typhimurium, Shigella flexneri* and *Chlamydia trachomatis* did not substantially induce IFN-lambda. We also found that IFN-lambda genes were up-regulated in lung epithelial cells infected with *Mycobacterium tuber-*

culosis and in hepatocytes and trophoblastic cells infected with *L. monocytogenes*. In a humanized mouse line permissive to fetoplacental listeriosis, IFN-lambda mRNA levels were enhanced in placentas infected with *L. monocytogenes*. In addition, the feto-placental tissue was responsive to IFN-lambda. Together, these results revealed that IFN-lambda may be an important modulator of the immune response to Gram-positive intracellular bacteria in epithelial tissues (Bierne et al. 2012).

The demonstration of the impact of lactobacilli on oral infection by Listeria

A comprehensive analysis of the impact of two *Lactobacillus* species on *L. monocytogenes* and orally acquired listeriosis in a gnotobiotic humanized mouse model was performed. We first assessed the effect of treatment with each *Lactobacillus* on *L. monocytogenes* counts in host tissues, and showed that each decreases *L. monocytogenes* systemic dissemination in orally inoculated mice. A whole genome intestinal transcriptomic analysis revealed that each *Lactobacillus* changes expression of a specific subset of genes during infection, with IFN-stimulated genes (ISGs) being the most affected by both lactobacilli. We also examined microRNA (miR) expression and showed that three miRs (miR-192, miR-200b and miR-215) are repressed during *L. monocytogenes* infection. Treatment with each *Lactobacillus* increased miR-192 expression, whereas only *L. casei* association increased miR-200b and miR-215 expression. Finally, we showed that treatment with each *Lactobacillus* significantly reshaped the *L. monocytogenes* transcriptome and up-regulated transcription of *L. monocytogenes* genes encoding enzymes, allowing utilization of intestinal carbon and nitrogen sources, in particular genes involved in propanediol and ethanolamine catabolism and cobalamin biosynthesis. Altogether, these data reveal that the modulation of *L. monocytogenes* infection by treatment with lactobacilli correlates with a decrease in host gene expression, in particular ISGs, miR regulation, and a dramatic reshaping of *L. monocytogenes* transcriptome (Archambaud et al. 2012).

The identification of an NK cells subset critical for Listeria *infections (coll E. Vivier)*

The complexity of gut cells that participate to mucosal immunity has recently emerged via the identification of several subsets of innate lymphoid cells (ILCs). In the framework of a collaboration with E. Vivier's group, we have shown that oral *Listeria* infection induces IFN-lambda production in small intestine NKp46 ROR-gamma-t- ILCs (or SI NK cells) and IL-22 production in NKp46+ ROR-gamma-t+ ILCs. However, only IFN-lambda SI NK cells contribute to control bacteria dissemi-

nation. The situation with *Listeria* is thus different from infection with other bacteria, e.g., *Citrobacter rodentium,* in which IL-22 plays a role in the control of infection (Reynders et al. 2011).

The elucidation of a transcytosis phenomenon in globlet cells during infection (coll M. Lecuit)

L. monocytogenes crosses the intestinal barrier upon interaction between its surface protein InlA and its species-specific host receptor E-cadherin (Ecad). Ecad, the key constituent of adherens junctions, is typically situated below tight junctions and therefore considered inaccessible from the intestinal lumen. The study led by G. Nikitas in M. Lecuit's lab – cited here because of its important follow up of our previous studies – showed that Ecad is luminally accessible around mucus-expelling goblet cells (GCs), around extruding enterocytes at the tip and lateral sides of villi, and in villus epithelial folds. Upon preferential adherence to accessible Ecad on GCs, *Listeria* is internalized, rapidly transcytosed across the intestinal epithelium, and released in the lamina propria by exocytosis, from where it disseminates systemically. Thus, Listeria exploits intrinsic tissue heterogeneity to reach its receptor and has revealed transcytosis as a novel and unanticipated pathway that is hijacked by *Listeria* to breach the intestinal epithelium and cause systemic infection (Nikitas et al. 2011).

Main references

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Research programme

Our future research programme is based on our long lasting and deep knowledge of the bacterium *Listeria monocytogenes*, a paradigm for the study of intracellular parasitism and crossing of host barriers by pathogens, as well as an exceptional model in cell biology, and on the unique tools that we have generated, including transgenic and knock-in mice and a *Listeria* transcriptome browser (http://www.weizmann.ac.il/molgen/Sorek/listeria_browser; et al. 2012). We will continue to use *Listeria* as a model organism to explore new landmarks and horizons in microbiology, cell biology and infection biology.

L. monocytogenes is responsible for severe human foodborne infections. During infection, it disseminates from the intestine to the brain and placenta, after crossing three host barriers, i.e., the intestinal, blood-brain or feto-placental barriers. In most tissues, it is intracellular as it survives in macrophages and invades non-phagocytic cells. *Listeria* then spreads from cell to cell using a process based on actin polymerization at one pole of the bacteria. Several virulence factors and key strategies used by *Listeria* for entry into cells, intracellular life, resistance to innate immune mechanisms and crossing of host barriers during infection have now been characterized in detail. An important step was reached when the genome of this organism and that of the non-pathogenic species *Listeria innocua* were sequenced. *Listeria* has become a multifaceted model, and general concepts in biology have emerged from studies on this organism. Yet, many aspects of the infection have not been examined: many bacterial components and host cell organelles have not been considered, nor have many signaling pathways been addressed. Our analysis should unearth unsuspected concepts in different fields.

We will investigate the infection at several levels: bacterial, cellular, and in many cases, at the tissue and organismal levels, in a spatio-temporal fashion. For several aspects, results obtained with *Listeria* will be challenged in other bacterial systems. Our project themes concern several emerging fields of research and will also pioneer new horizons. Some have been initiated. Others will be launched.

Our project is interdisciplinary in nature as it encompasses the fields of fundamental microbiology (regulation), cell biology, cellular microbiology, innate immunity and epigenetics. It relies on innovative techniques and sophisticated approaches. In all aspects, it is at the forefront of international research, and has four aims:

Aim1: Towards the discovery of novel RNA-mediated regulations

- CRISPRs, Antisense RNAs, riboswitches and small RNAs
- Internal transcription and translational start sites
- Small open reading frames (orfs)
- Secretion of small RNAs

Aim 2: Identification of novel virulence factors and virulence mechanisms

Aim 3: Investigating unexplored facets of the cell biology of infection

- Genome-wide approaches to analyze the successive steps of infection
- Mitochondria and mitochondrial dynamics in infection
- Post-translational modifications in the infected cell: SUMOylation, ISG15, Nedd8, Proteolysis, Acetylation

Aim 4: Exploring epigenetic reprogramming during infection

- Histone modifications
- DNA methylation
- Bacterial and host factors involved in chromatin regulation
- Inheritance of epigenetic marks

Together, the diverse and highly complementary facets of this proposal should generate a series of innovative contributions to microbiology, cell biology and infection biology.

Questions and Comments

Peter Meier-Abt

The floor is now open for discussion. May I ask the first question?

This has been a wonderful exposition, and it deals with a subject I have been very interested in: mitochondria. You describe mitochondria fragmentation – when this bacterium runs around within the cell, and then the mitochondria unite again and the cell is ok. How does this function?

Pascale Cossart

People generally don't know that mitochondria, in fact, are the places, the structures in the cell where there is production of ATP and energy. I was fascinated by mitochondria because they are supposed to be of bacterial origin, and one day I was giving a talk after someone who said mitochondria make filaments. I was not aware of that. And indeed mitochondria do make filaments, which are fusing or fragmenting

constantly. This is really essential for organism survival, and it correlates with mitochondrial function.

Upon infection, the mitochondria, which are filamentous, fragment, and this is amazingly rapid. Fragmented mitochondria do not make ATP for a short period – the cell is somehow anesthetized for a while. This is a transient phenomenon, and then the mitochondria re-fuse again, making ATP. We are studying this phenomenon. What is interesting is that the phenomenon is atypical. Indeed, we recently published a paper in PNAS a few months ago reporting this atypical fragmentation, a new phenomenon involving molecules that we are trying to identify. We have shown that it is the secreted bacterial protein which is called Listeriolysin O which is responsible for the phenomenon of mitochondrial fragmentation.

Peter Meier-Abt

Thank you very much – truly fascinating. I have a brief follow up question. This is a very complex mechanism, and this bacterium is running around in the cell. But if we have to develop a treatment which is effective, where would you interfere in this very complex cell biological mechanism? Before the *Listeria* goes into the cell?

Pascale Cossart

The problem with *Listeria* and listeriosis is not really the treatment, because most *Listeria* strains are still sensitive to antibiotics. We are lucky that the situation is not too bad in this respect. One can treat listeriosis with antibiotics, but as *Listeria* bacteria are intracellular, consequently one has to treat patients for a long period and with relatively high doses of antibiotics. Generally, we use two antibiotics. The main problem with *Listeria* is the diagnosis, which has to be made as soon as possible, because when the bacteria reach the brain, they may induce neurological sequelae. So what is really needed is an early diagnosis to prevent these negative consequences. To answer your question more directly, therapy treatment is not a real problem for *Listeria* for the moment. But, as I have indicated, one approach to block the infectious process could be to block the thermosensor. It would be a totally new biochemical approach.

Peter Suter

Why is the affinity of *Listeria* to the brain, the liver and some other organs so much stronger, or are the effects simply more visible there than in the heart and in the lungs?

Pascale Cossart

Listeria is a bacterium which, once it has crossed the intestinal barrier, is found in the blood; then it is filtered somehow in the liver. The tropism for the placenta, for example, as we have shown, is mainly due to the fact that the trophoblastic cells express E-cadherin, a protein which has a high affinity for the bacterial protein internalin. So, via an interaction between internalin which is expressed on the surface of the bacterium and E-cadherin, the bacteria can adhere and then cross the placental barrier. For the brain, we don't know for the moment why there is a tropsim for this organ.

Heinz Gutscher

You are very keen on therapeutics. I have a question about prevention. You showed this beautiful picture of French cheese which is not pasteurized. Now my question is whether there is a technique to avoid *Listeria* from spreading in cheese or are they always there and it is just a question of the dose?

Pascale Cossart

Well, what you say is true. *Listeria* is present in many places in the environment and can contaminate many food products. Of course if there are very few bacteria, there is no problem. But if the dose is higher and the chances are higher in a cheese which is not pasteurized, then the chances of getting infected are higher. One has to remember that each individual is not sensible to the same dose of bacteria. The status of the immune system is critical and the elderly are clearly more sensitive than adults.

Question from the audience

Are there other pathogens using the same adhesion molecules, i.e., the E-cadherins?

Pascale Cossart

There is a report indicating that *Candida albicans* which is not a bacterium – it's a fungus – can also use E-cadherin. So far, I don't know any other. E-cadherin is an ideal molecule to bind to! It has a transmembrane domain, and via its cytoplasmic domain it interacts with the cytoskeleton. So – and this is amazing – before we found it as being involved in *Listeria* adherence and entry into cells, I had a discussion with researchers working on E-cadherins, and had said that this protein would be a very good candidate. It happened to be the case, but I think what is particularly

interesting with human E-cadherin is its strong and specific interaction with internalin, while the murine E-cadherin, which is more than 96% similar to the human, does not interact with internalin. We have succeeded to mutate one single amino acid in the murine E-cadherin (a glutamic acid into a proline) and showed that this change led to a strong affinity for the internalin. The co-crystal that I presented to you was not obtained by us, but by a group in Braunschweig, which highlighted that this amino acid was protruding from the surface of E-cadherin and prevented the interaction with internalin, demonstrating how host specificity is achieved at the molecular level.

Peter Meier-Abt

Regarding the exit from the cell, is this a passive mechanism, or is it because the cells are dying and the bacteria just leaves?

Pascale Cossart

Bacteria don't really exit from the cell, they spread from one cell to the other. It's not what is observed with *Legionella* or *Chlamydia*. For these two bacteria, replication occurs in the first infected cell, bacteria divide, then the cell bursts out. For *Listeria*, bacteria multiply in the cytosol, prevent the cell death and spread from one cell to the other.

Peter Meier-Abt

Thank you again for this fascinating account, and congratulations again on winning the Balzan Prize. The next prizewinner will now be introduced by Luciano Maiani, who is also a member of the Balzan General Prize Committee.