«Les multiples stratégies d'une bactérie pour infecter et persister dans son hôte »

Pascale COSSART

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Infectious diseases are a major health problem

They are responsible for 15 millions deaths per year (26% of annual deaths worldwide):

- Respiratory diseases : 3.9 millions
- HIV : 2.9 millions
- Diarrheal diseases : 2 millions
- Tuberculosis : 1.6 millions
- Malaria : 1.1 millions

Some cancers are associated with an infectious agent

15 to 20 % of cancers are caused by an infectious agent (a virus or a bacterium)

- gastric cancer by *Helicobacter pylori*
- liver cancer by hepatitis B virus
- cervic cancer by papilloma virus

Emergence and re-emergence of infectious diseases

- **Old diseases** are re-emerging : in many cases (e.g.tuberculosis), they are more virulent due to **multi-drug resistance**

- **New diseases** are emerging due to major changes in socio-economical , ecological, and climatic conditions.
  Species barriers are crossed and animal diseases (in most cases, due to viruses or prions) can affect humans (Avian flu…)

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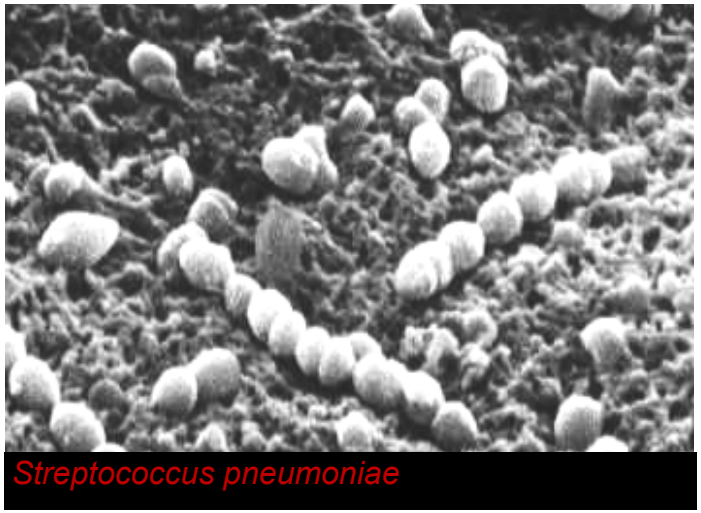
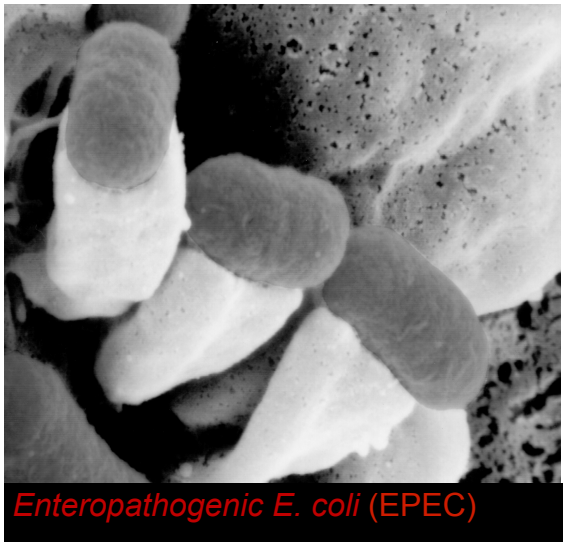
Urgent need for new therapeutics

A full understanding of infectious processes
is an absolute prerequisite
for the design and generation of new anti-infection drugs

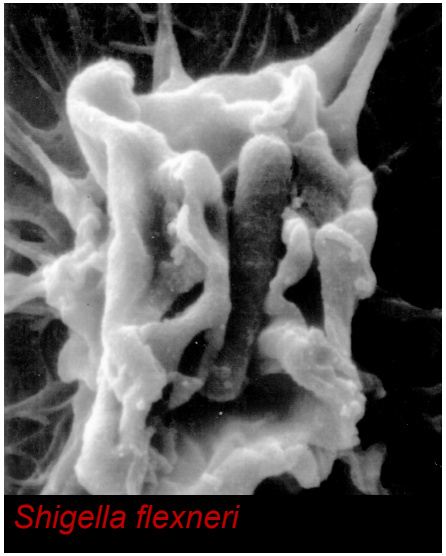
Extracellular and intracellular pathogens



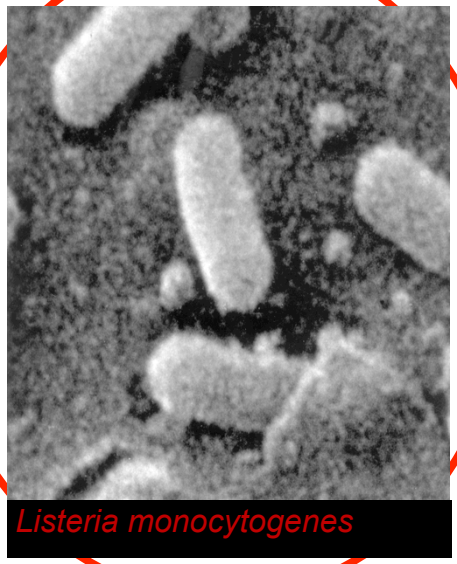
Streptococcus pneumoniae



Enteropathogenic E. coli (EPEC)

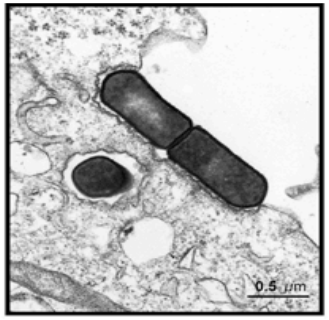


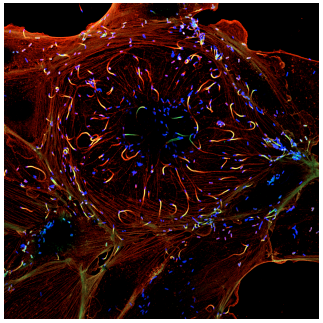
Shigella flexneri

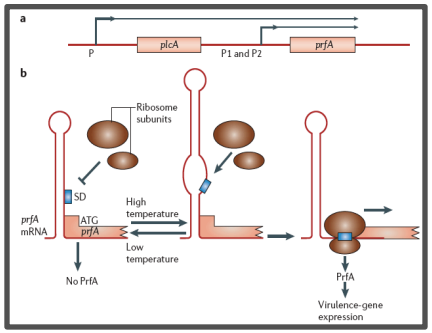


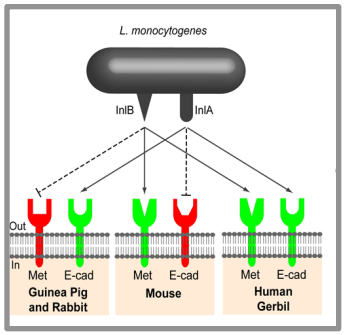
Listeria monocytogenes

Legionella pneumophila

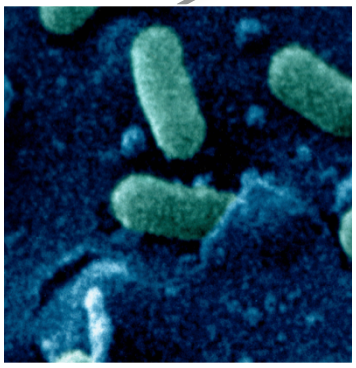


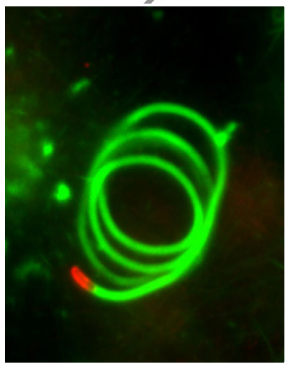


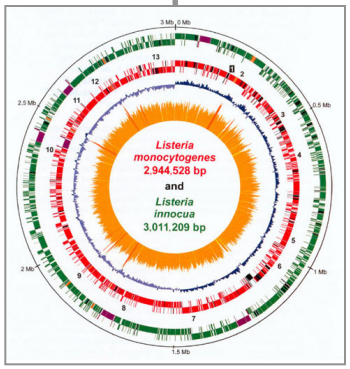


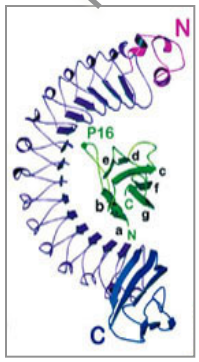


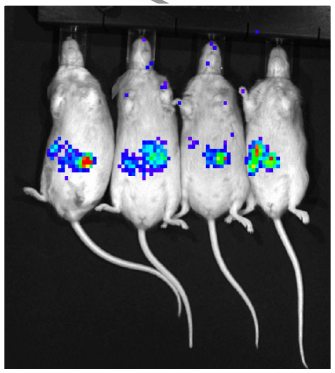
Listeria monocytogenes
1986-……











- Genetic approaches coupled to cell biology and molecular biology

  « Cellular Microbiology »*
- Post-genomic approaches
- Hypothesis-driven approaches

Cellular Microbiology Emerging

P. Cossart, P. Boquet, S. Normark, R. Rappuoli

A new discipline, cellular microbiology, is emerging at the interface between cell biology and microbiology. Traditional cell biological approaches are already widely used to unravel the tactics microbes utilize to infect their hosts, but the use of pathogens to tackle questions in cell biology is just now yielding promising approaches and elegant results. Two meetings, in 1989 and 1991 (*1*), laid the groundwork for the field, and a third meeting in 1995 highlighted recent progress (*2*).

A major focus of this new field is the actin network, which together with intermediate filaments and microtubules constitute the cytoskeleton. The rapid assembly and disassembly of actin microfilaments is essential for phagocytosis, motility, cell division, and adhesion to a substratum or to another cell. Yet, the signaling pathways that control actin dynamics are poorly understood. Bacteria that can be genetically manipulated and parasites can provide tools to dissect these control pathways. When certain bacteria, such as *Salmonella* and *Shigella*, infect cells, they mimic the action of epidermal growth factor (EGF), inducing membrane ruffling and active actin polymerization (*3*–*5*) (see figure). The ruffling leads to internalization of the bacteria.

The internalization of other pathogens occurs without membrane ruffling or even actin polymerization. The parasite *Trypanosoma cruzi* enters cells by triggering a combination of events—a transient increase in cytosolic free calcium, rapid rearrangement of the cortical actin cytoskeleton, and lysosome recruitment and clustering at the invasion site (*6*, *7*). Lysosomes contribute membrane for the formation of the parasitophorous vacuole. Disruption of cortical actin by the increase in local calcium allows lysosomes to migrate and fuse, a phenomenon also regulated by calcium. Phospholipase C

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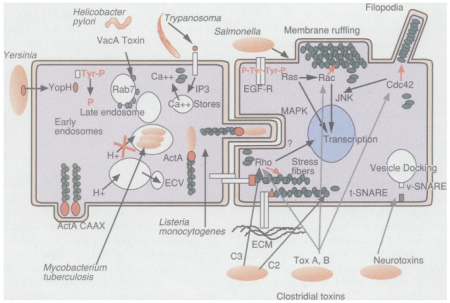
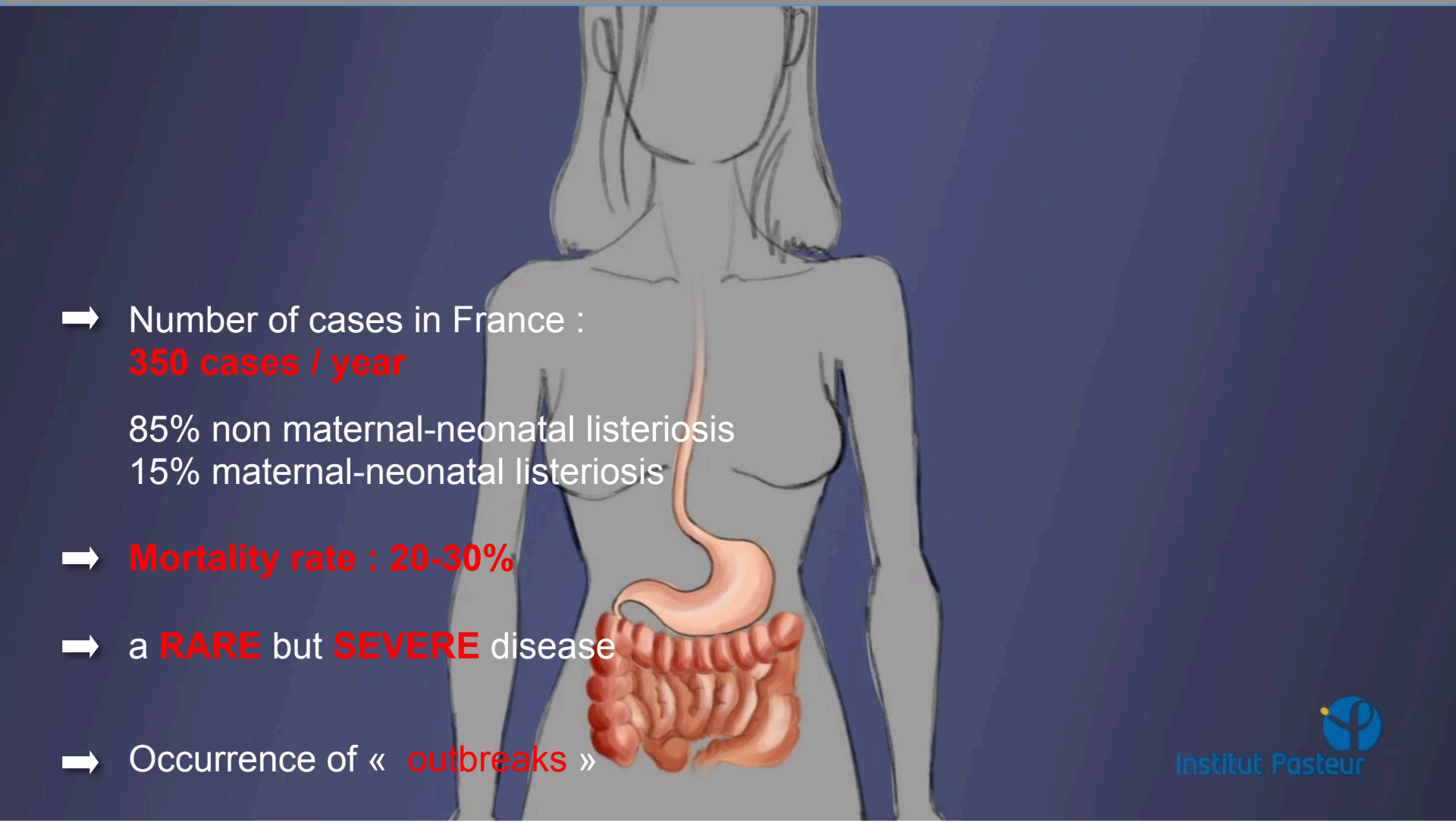


Pathogenic bacteria interfere with numerous eukaryotic cell functions, providing a sophisticated tool kit for cell biologists.

Listeriosis



- Number of cases in France :
  350 cases / year

  85% non maternal-neonatal listeriosis
  15% maternal-neonatal listeriosis

- **Mortality rate : 20-30%**

- a **RARE** but **SEVERE** disease

- Occurrence of « outbreaks »

Institut Pasteur

Listeria 2018 important outbreak in South Africa

Food Safety News
Breaking news for everyone's consumption

South Africa's processed meats blamed in Listeria outbreak

BY **DAN FLYNN** | MARCH 5, 2018

You might say the source of South Africa's deadly Listeria outbreak was hiding in plain sight, but it took the deaths of 180 people before the Health Minister could collect enough evidence to name the culprits.

A cold, processed meat product made by South Africa's biggest producer of consumer foods is the cause of the ongoing outbreak, which has a stunning 27 percent fatality rate.

Dr. Aaron Motsoaledi, South Africa's Minister of Health, on Sunday announced the source of the outbreak was two unrelated brands of "polony" manufactured by the Tiger Brands unit of Enterprise Foods and Rainbow Chicken Limited (RCL).

The minister said the Enterprise and RCL polony products are being recalled, and he went further.

"We advise members of the public to avoid all processed meat products that are sold as ready-to-eat," Motsoaledi said.

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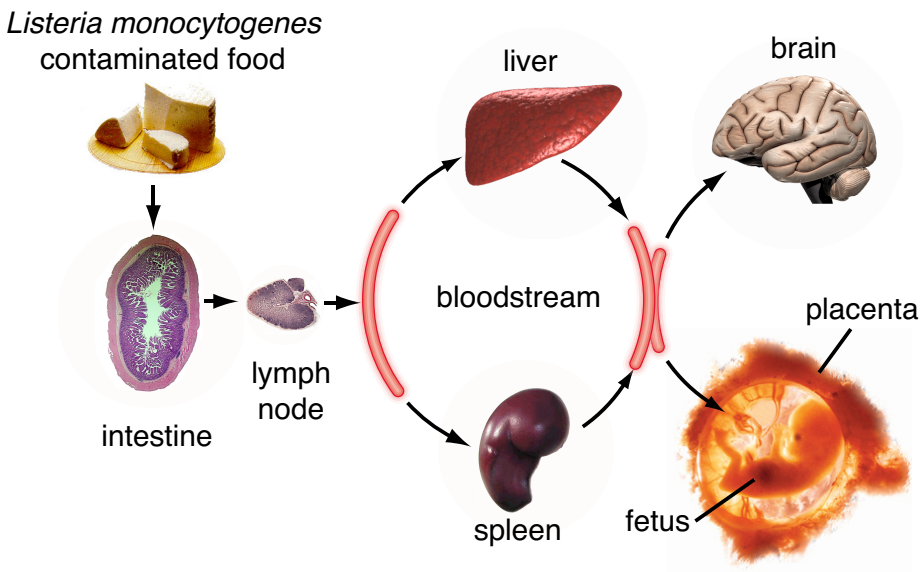
FOOD RECALLS

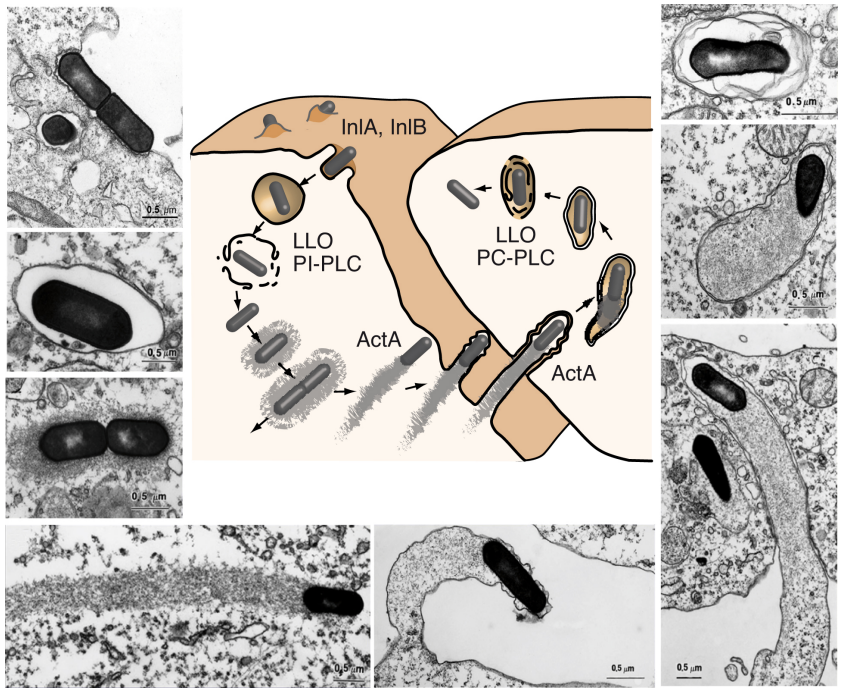
- Consumer complaints spur recall of canned chicken nationwide
- Canada's Salmonella investigation sparks chicken nugget recall
- Illnesses prompt testing, recall of deli roast beef in Canada
- Blue Ridge Beef recalls more raw pet food for bacteria

The Listeria infection process

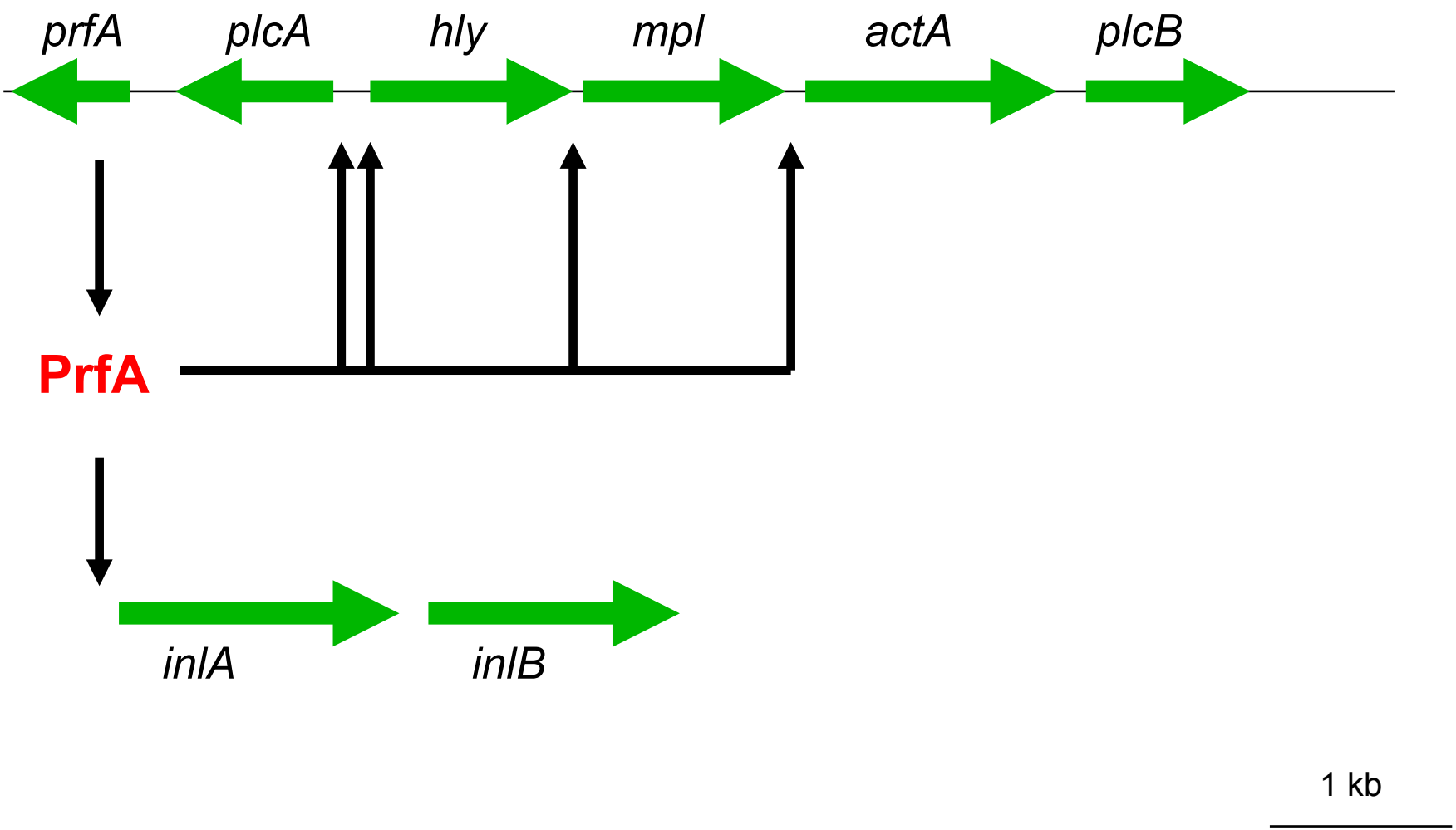
Successive steps of human listeriosis



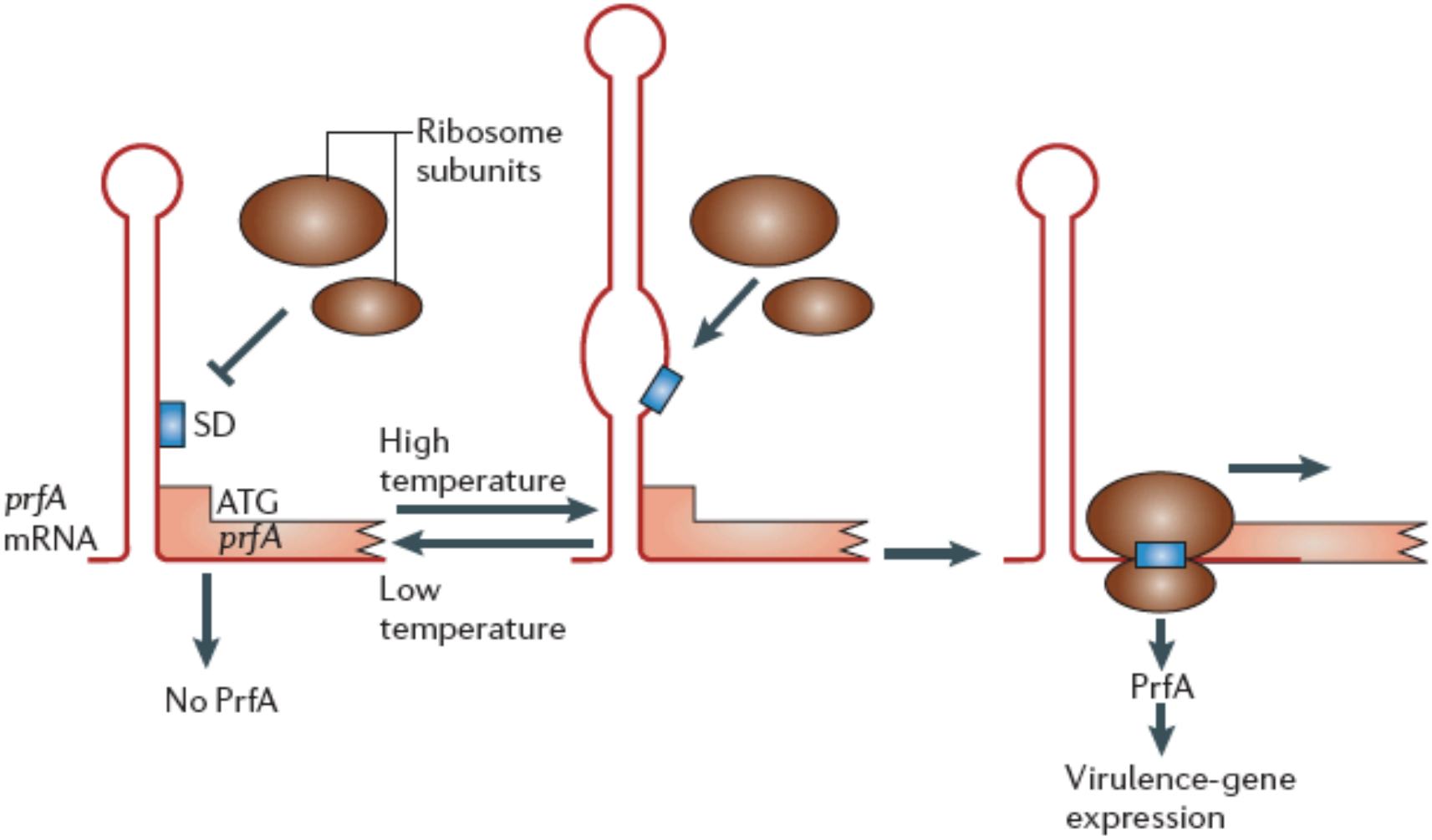
Cell infectious process



The *Listeria monocytogenes* virulence gene regulon



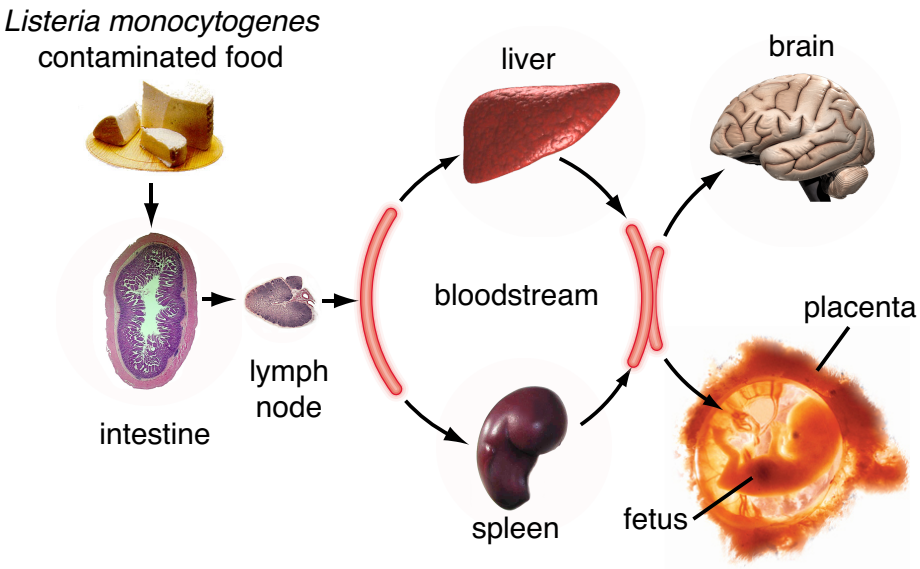
Thermoregulated expression of PrfA



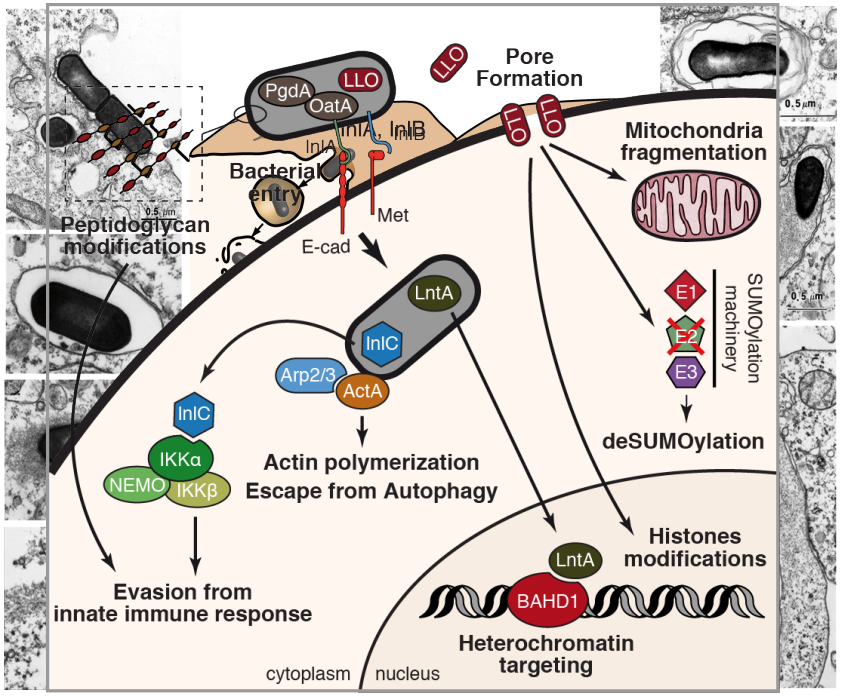
Johansson *et al.,* Cell 2002

The Listeria infection process

Successive steps of human listeriosis

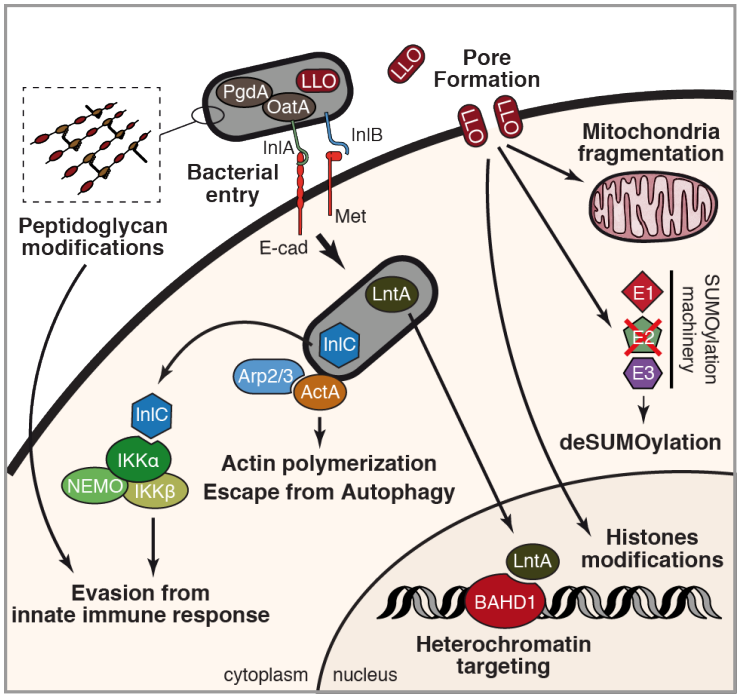


Listeria monocytogenes cell biology



Listeria monocytogenes: a model invasive pathogen

Listeria monocytogenes : a cell biologist

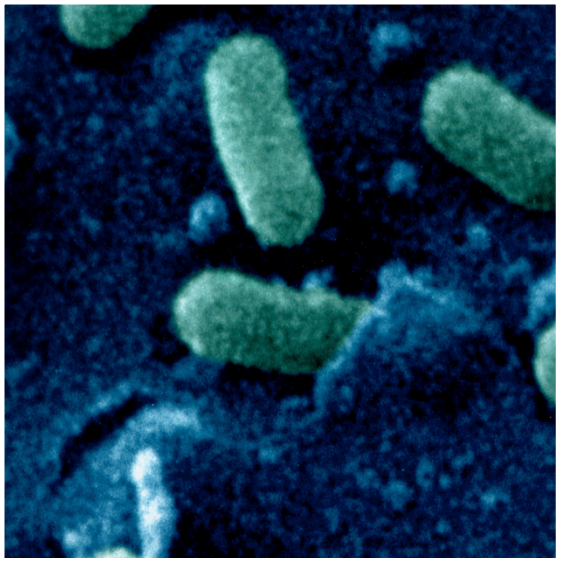


Listeria monocytogenes: entry into cells

Cell, Vol. 65, 1127–1141, June 28, 1991, Copyright © 1991 by Cell Press

Entry of L. monocytogenes into Cells Is Mediated by Internalin, a Repeat Protein Reminiscent of Surface Antigens from Gram-Positive Cocci

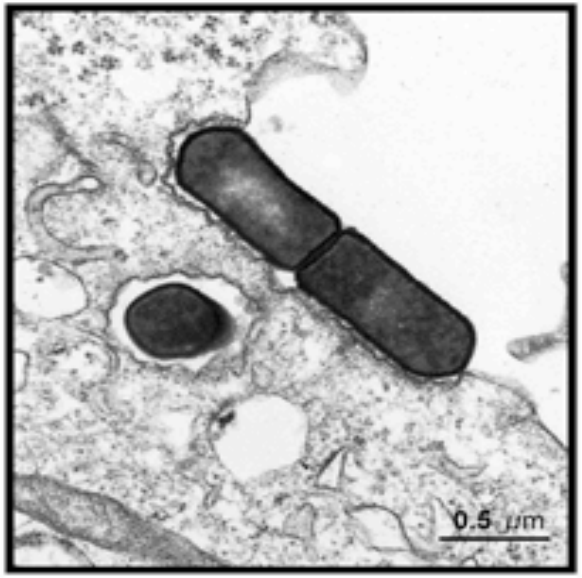
J.-L. Gaillard,* P. Berche,* C. Frehel,*
E. Gouin,† and P. Cossart†



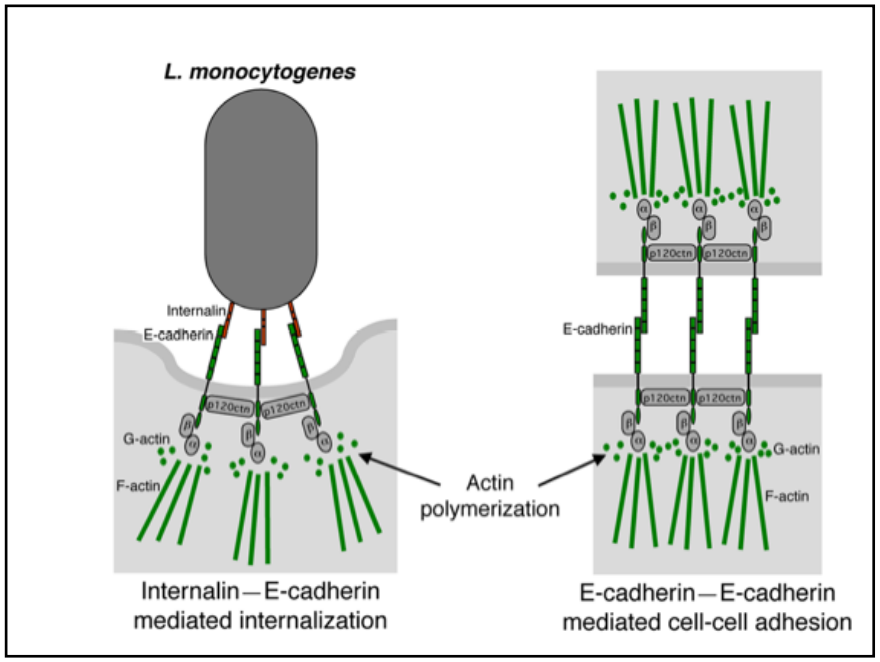
Mol Microbiol. 1995 Apr; 16 (2):251-61.

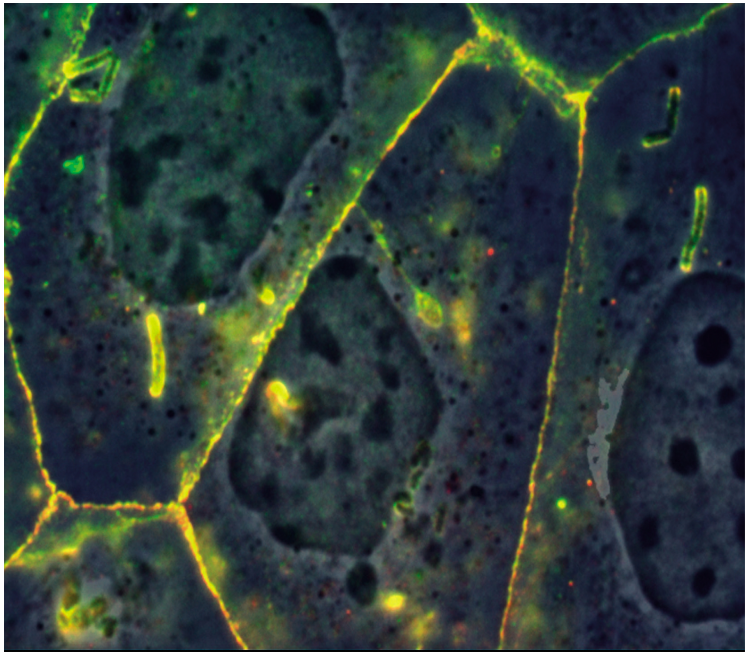
Entry of Listeria monocytogenes into hepatocytes requires expression of InlB, a surface protein of the internalin multigene family.

Dramsi S, Biswas, I, Maguin E, Braun L, Mastroeni P, Cossart P.

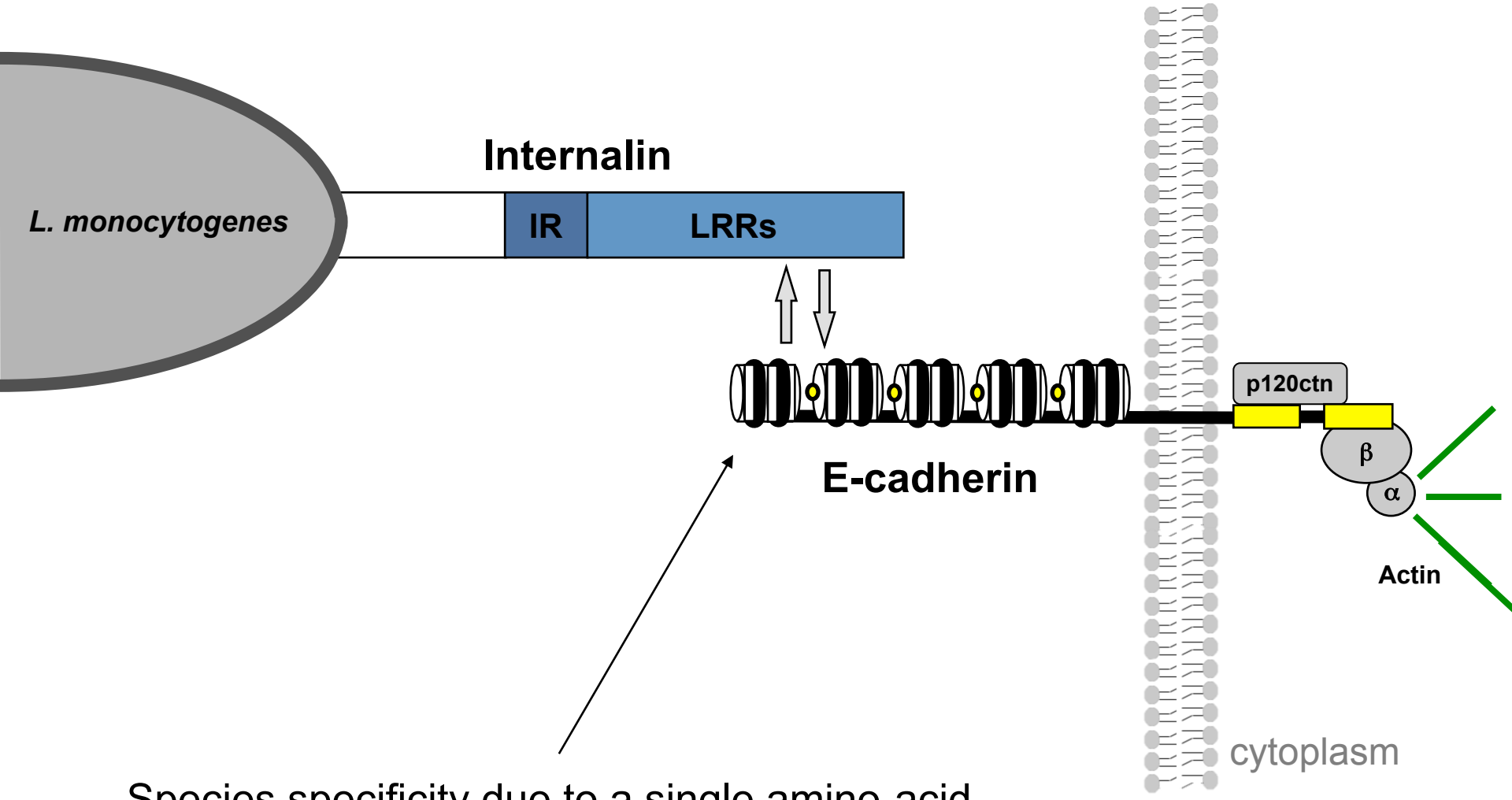


Listeria monocytogenes: entry into cells





The interaction between internalin and E-cadherin is species specific

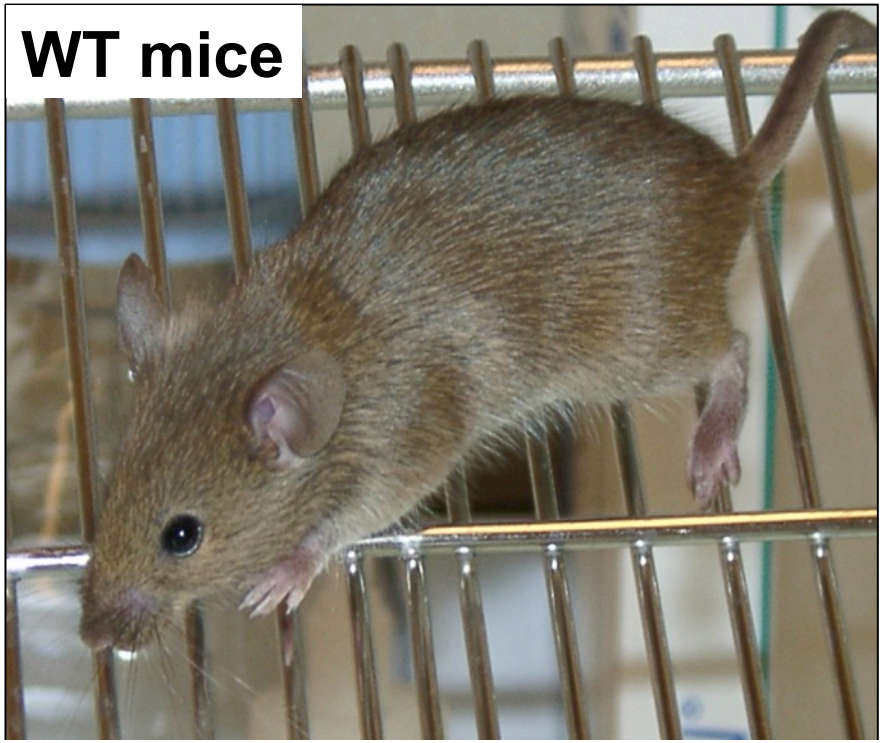


Species specificity due to a single amino-acid

Lecuit et al., EMBO J 1999

The first transgenic mouse model for Listeriosis

WT mice



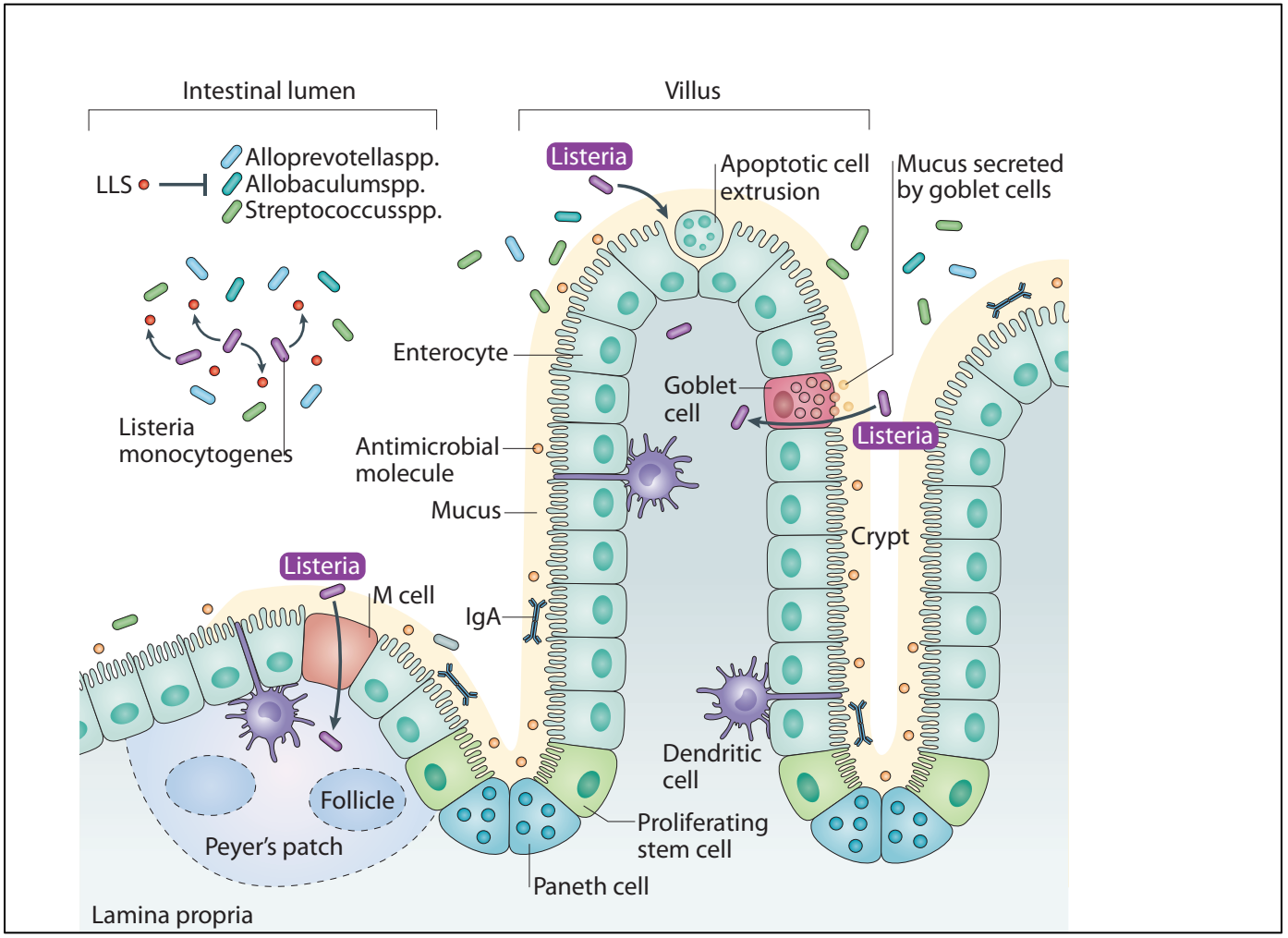
$5\ 10^{10}$	*L. monocytogenes* WT	0 %
$5\ 10^{10}$	Δ*inlA*	0 %

hEcad transgenic mice

$5\ 10^{10}$	*L. monocytogenes* WT	~ 100 %
$5\ 10^{10}$	Δ*inlA*	0 %

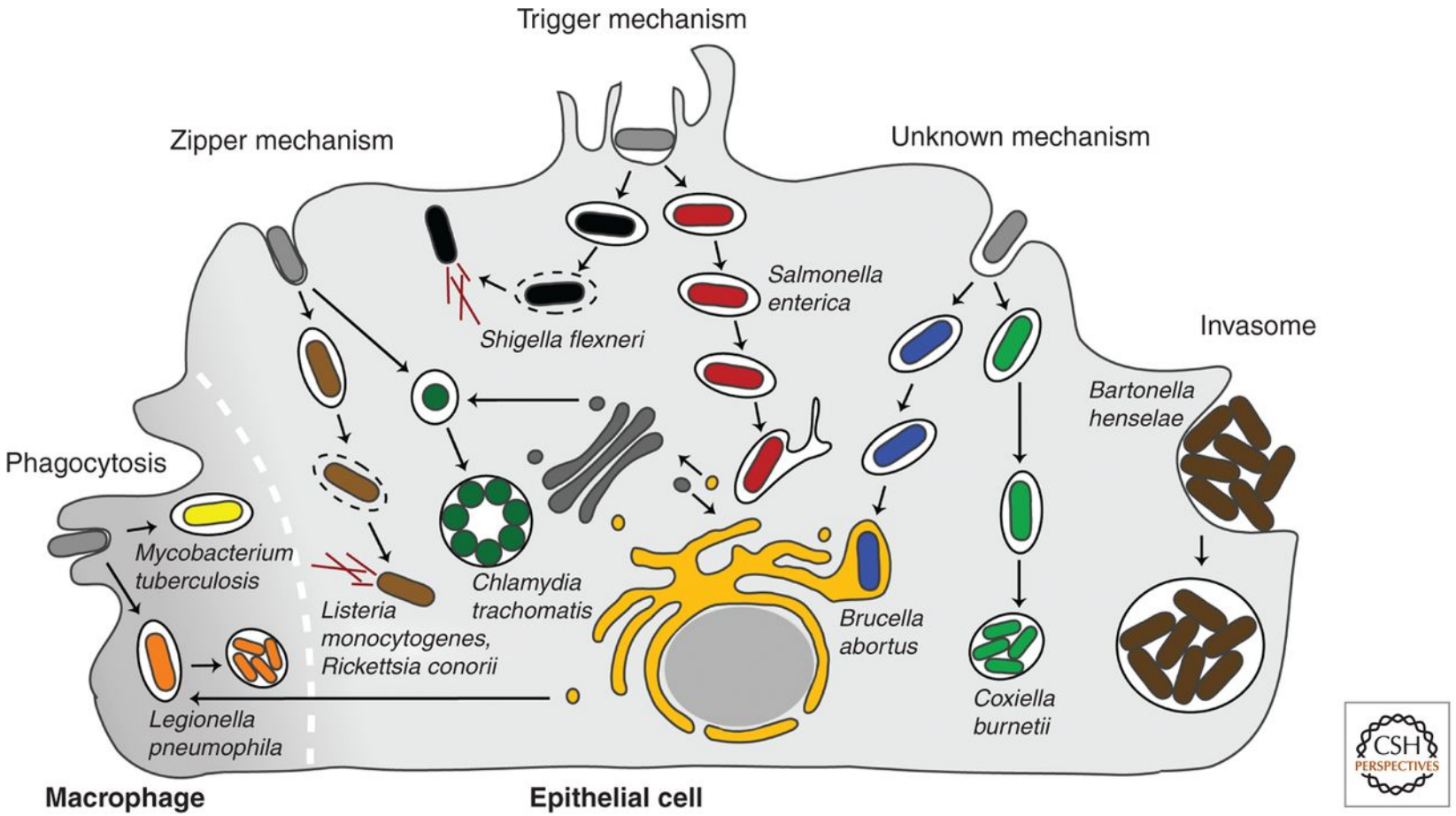
Lecuit et al., Science 2001

Crossing of the intestinal barrier



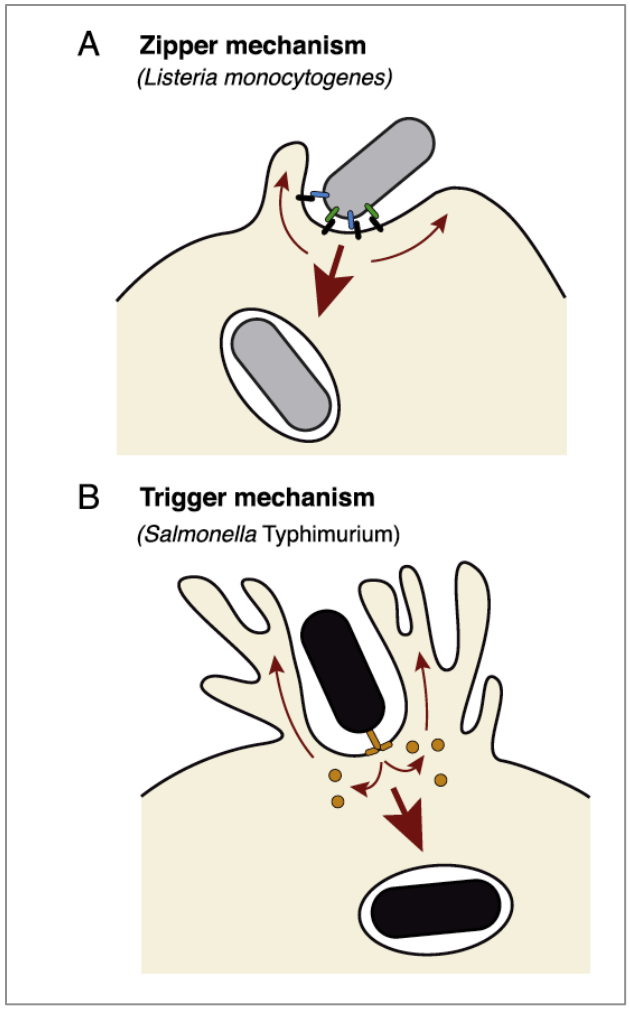
Radoshevich and Cossart (2018) Nature Microbiol.

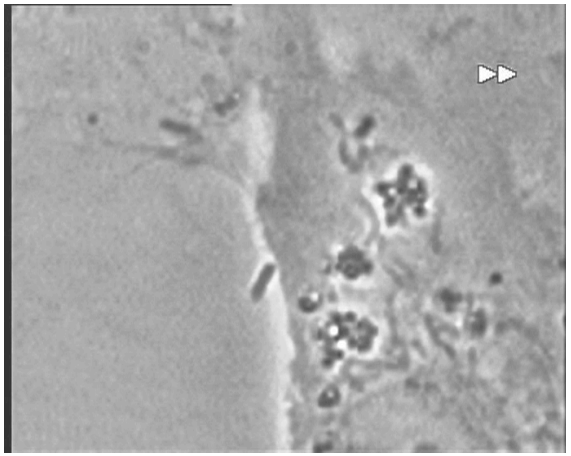
Intracellular bacterial pathogens

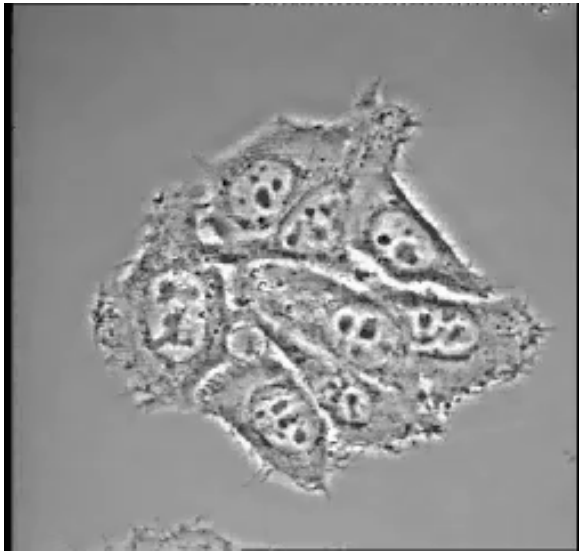


Cossart P , and Helenius A
Cold Spring Harb Perspect Biol 2014;6:a016972

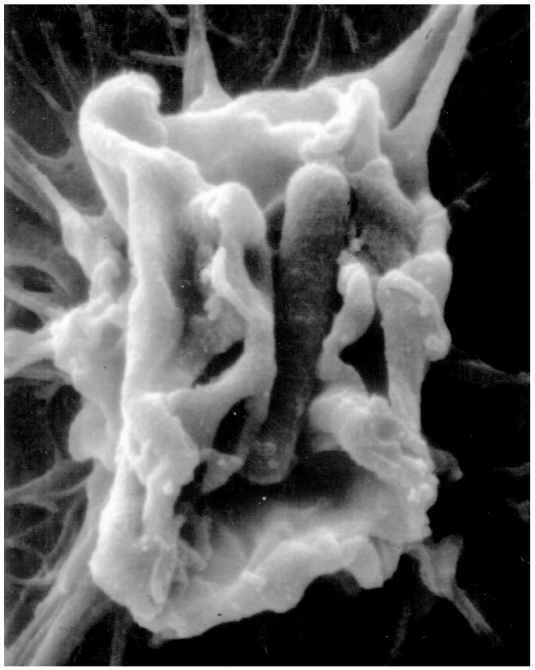
Entry into cells : the zipper and trigger mechanisms

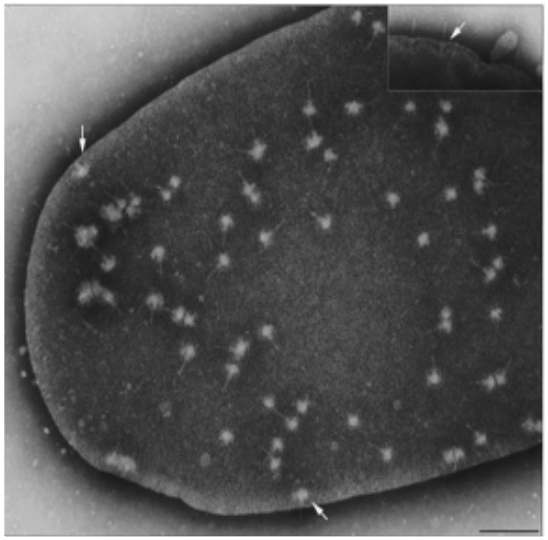


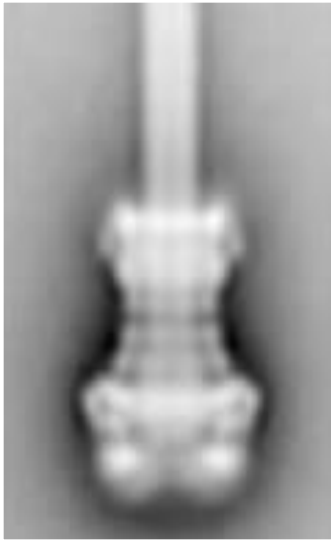


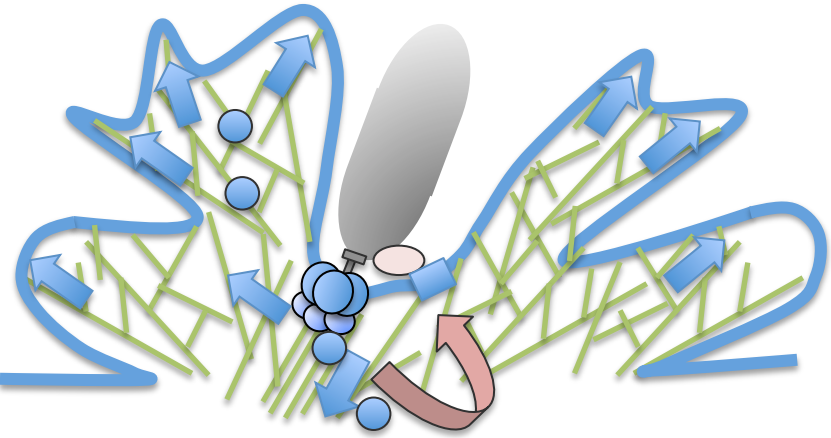


Shigella flexneri and the type III secretion system (T3SS)

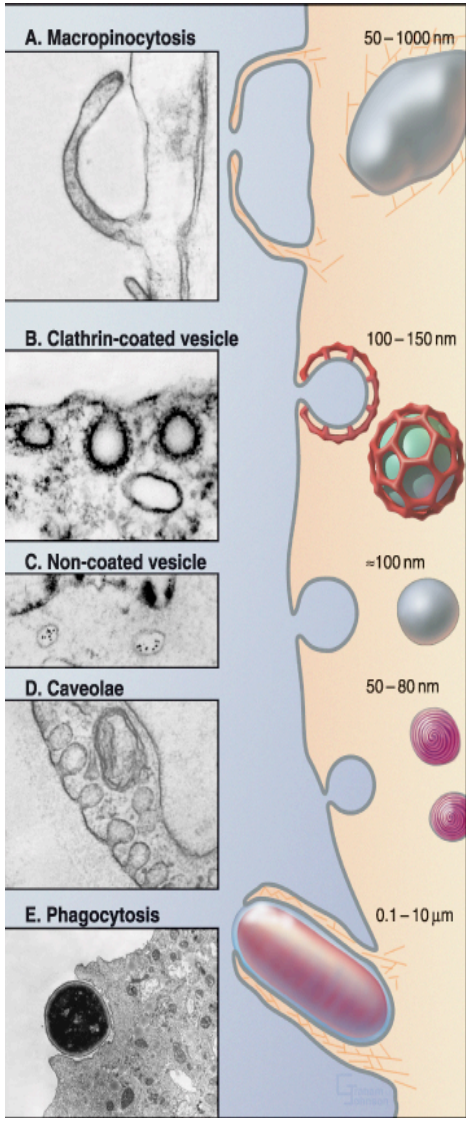


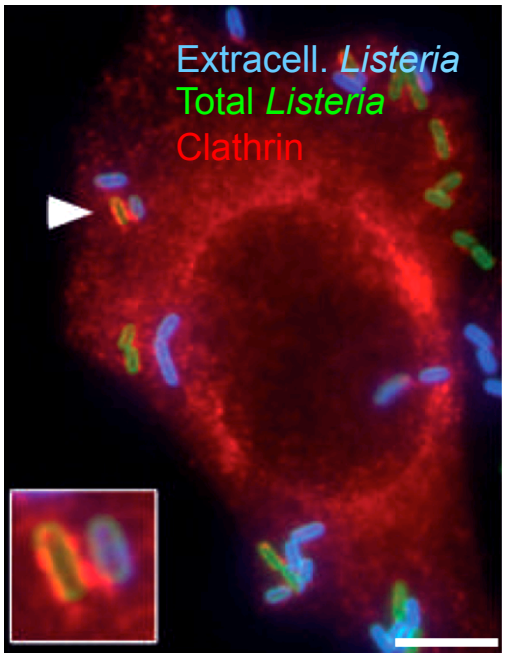


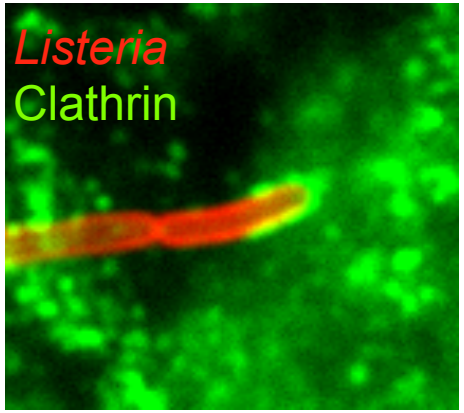




A shift in paradigm: a role for clathrin in phagocytosis



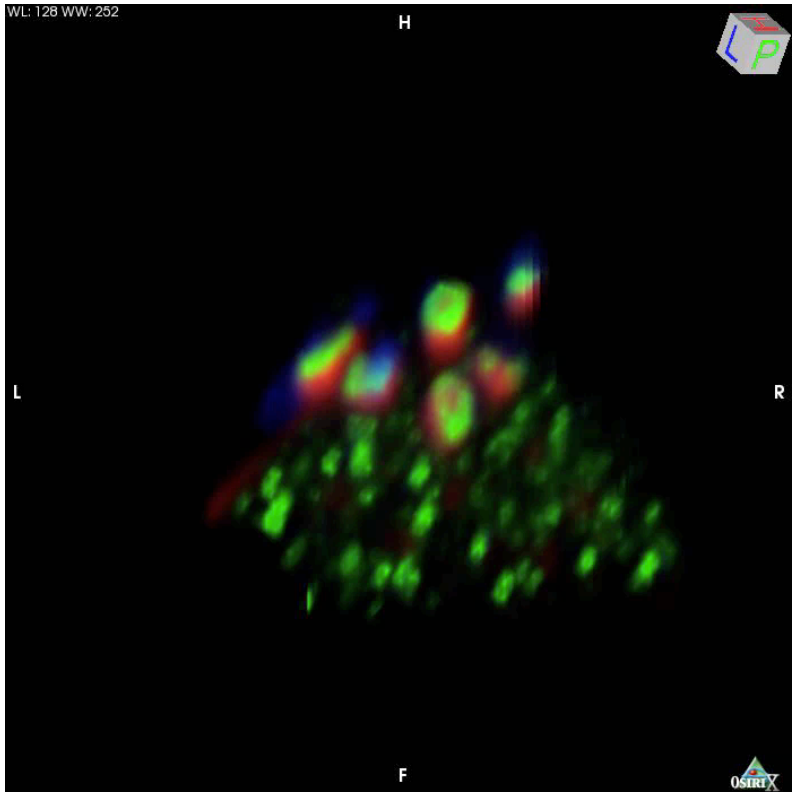




Clathrin is used for internalization of objects larger than previously accepted

Veiga and Cossart, Nat. Cell Biol. 2005 ; Veiga et al., Cell H & M, 2007; Bonazzi et al., Cell. Micro. 2008.

Clathrin is also used for adherence of extracellular bacteria

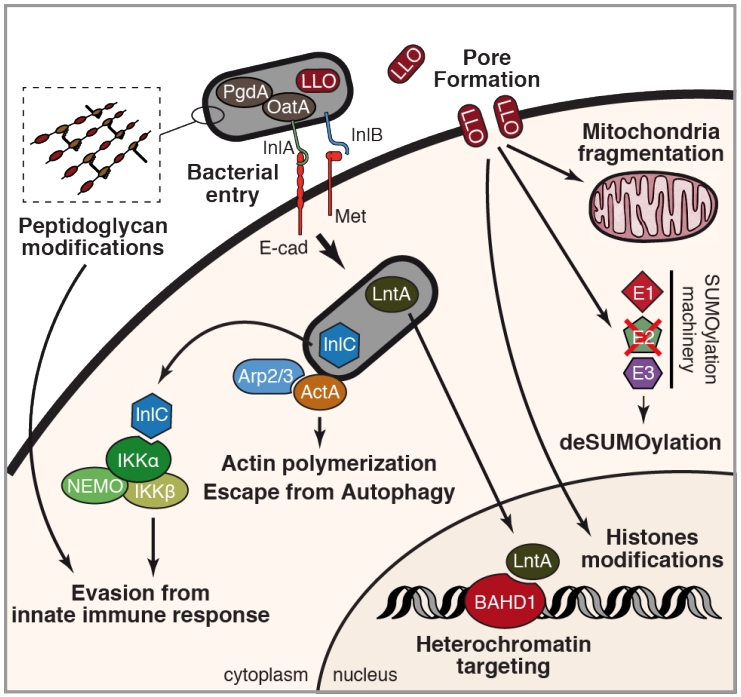


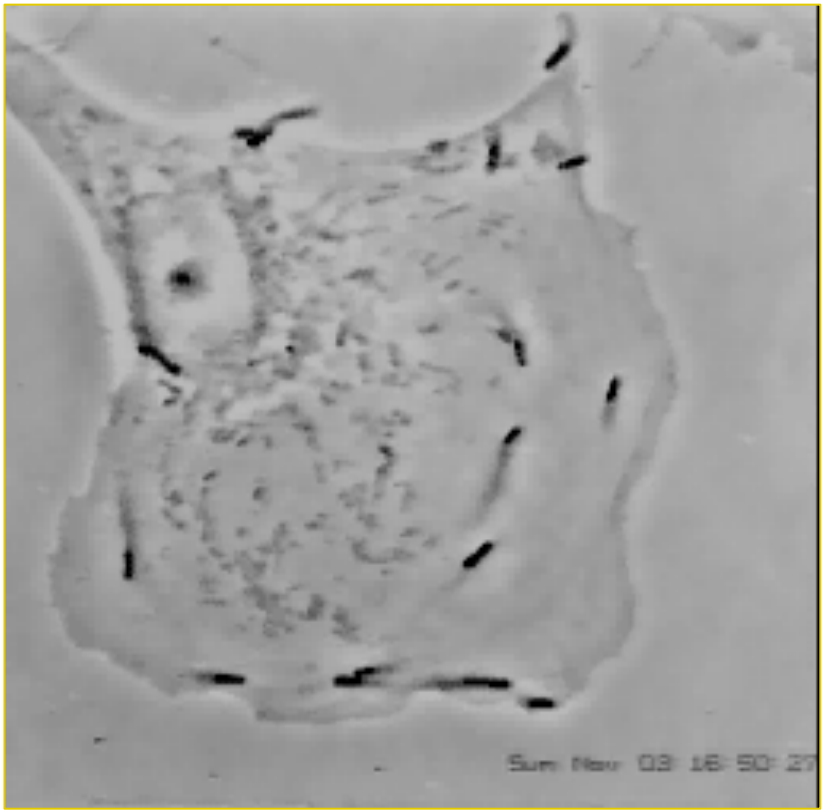
EPEC actin clathrin

Veiga et al., Cell Host & Microbe, 2007; Guttman et al., Infect. Immun. 2010

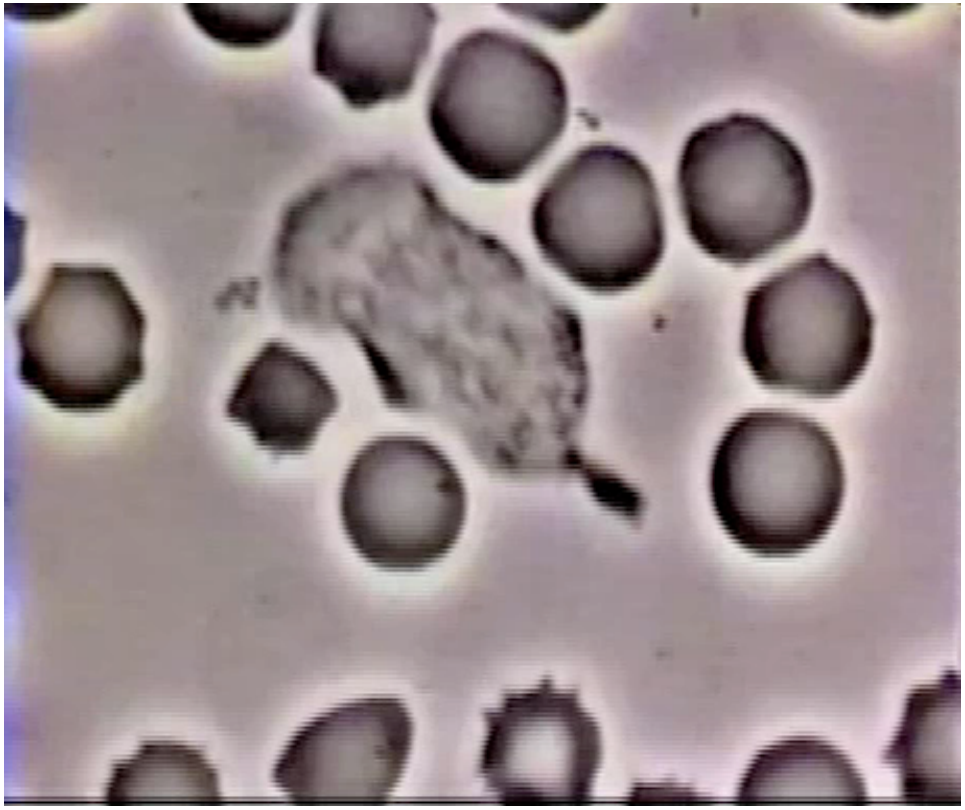
The Listeria actin-based motility

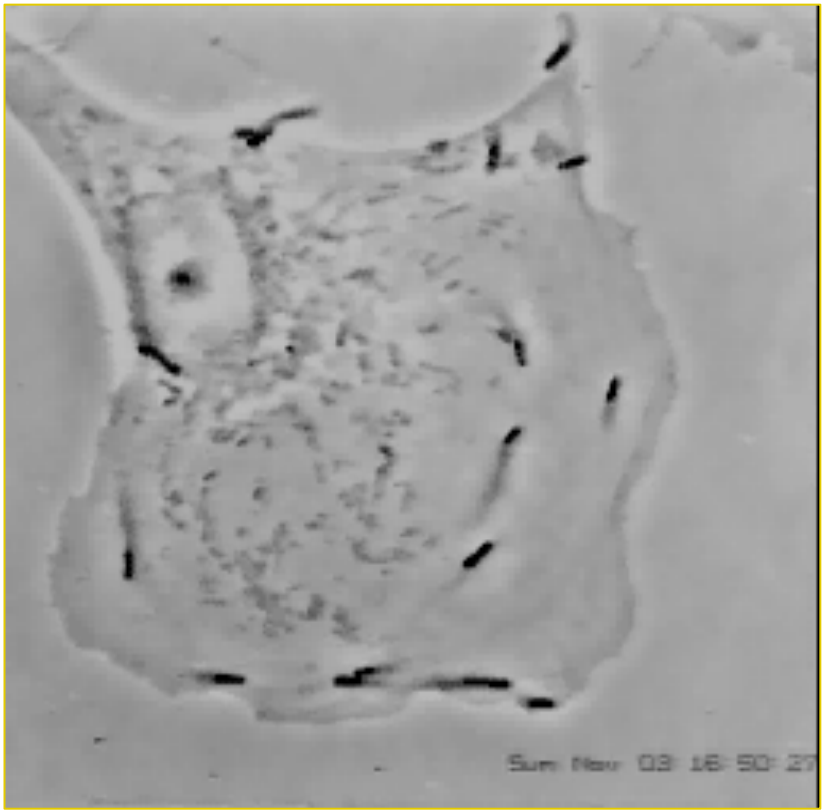
Listeria monocytogenes **: a cell biologist**



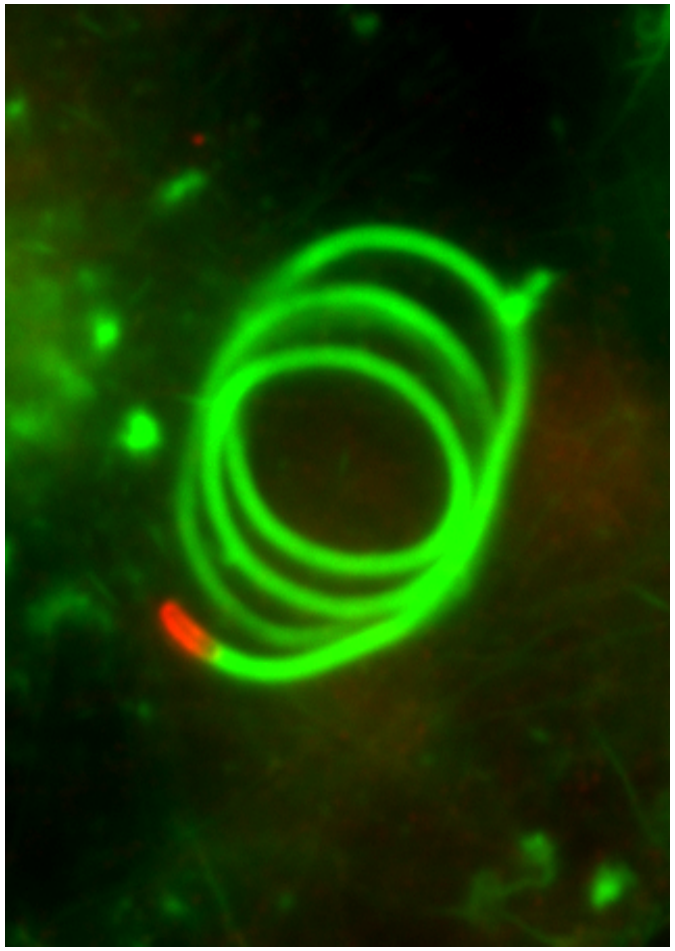


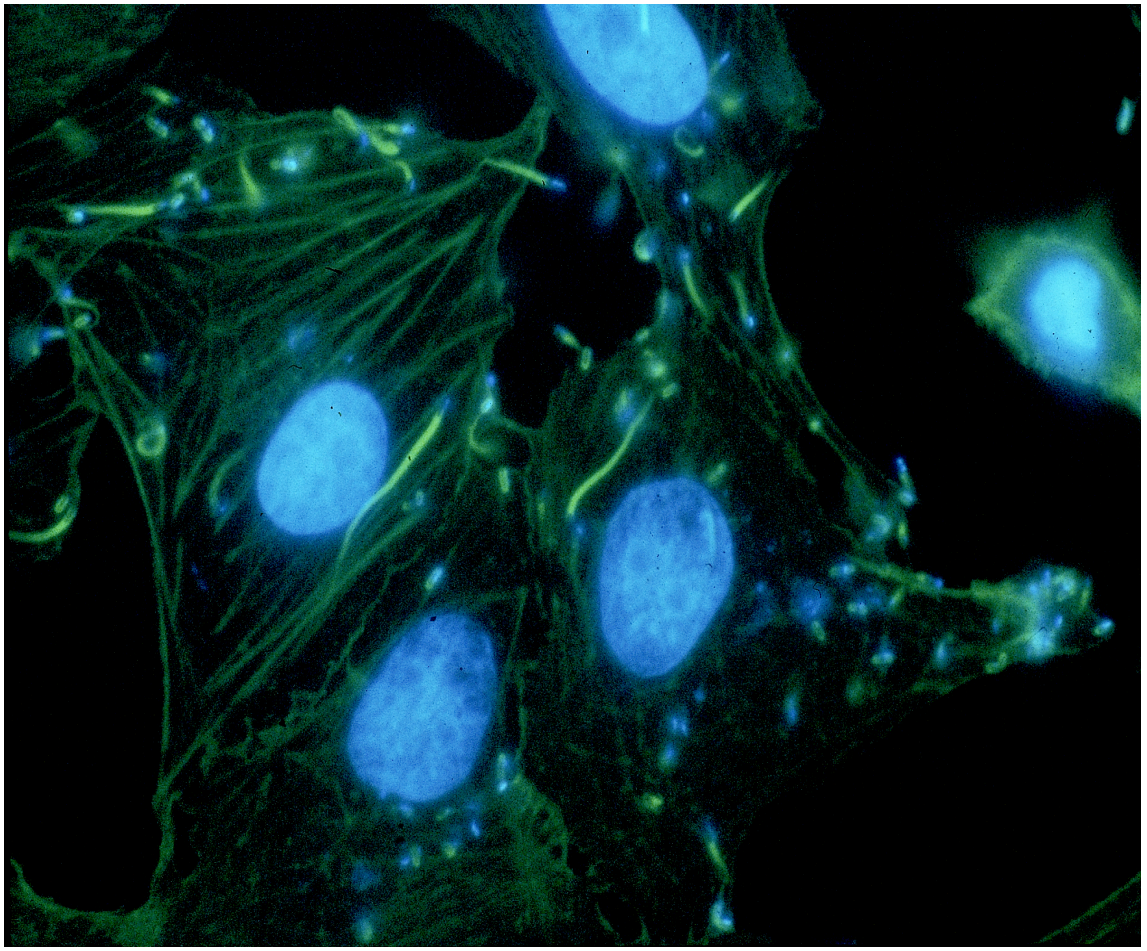
The Listeria actin-based motility



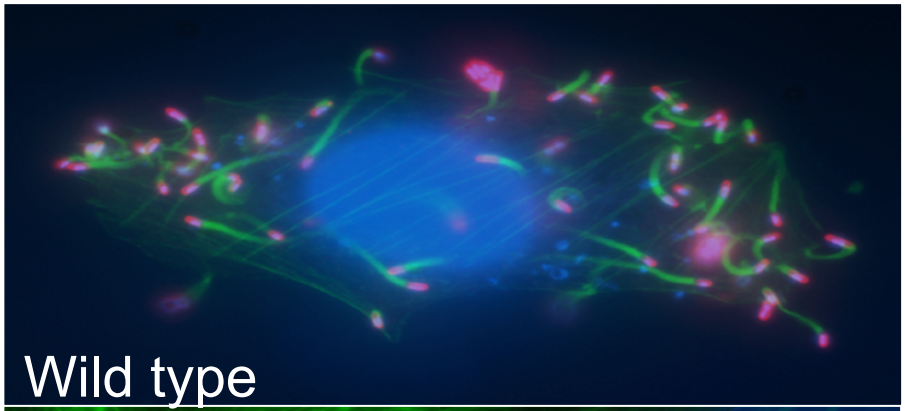


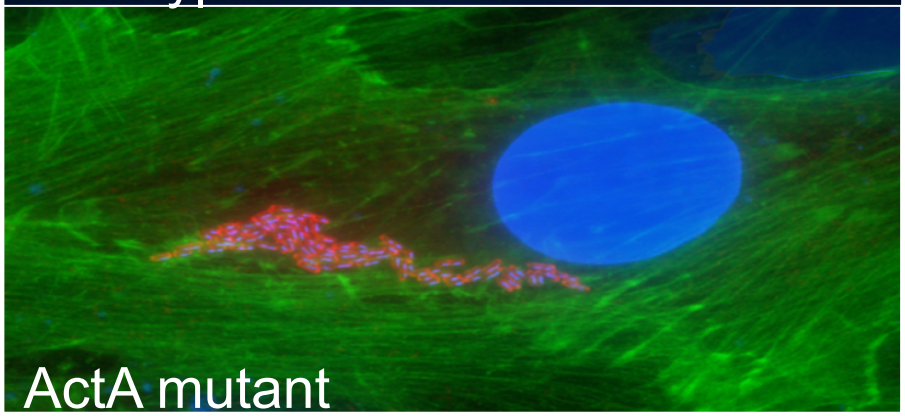
The Listeria actin-based motility

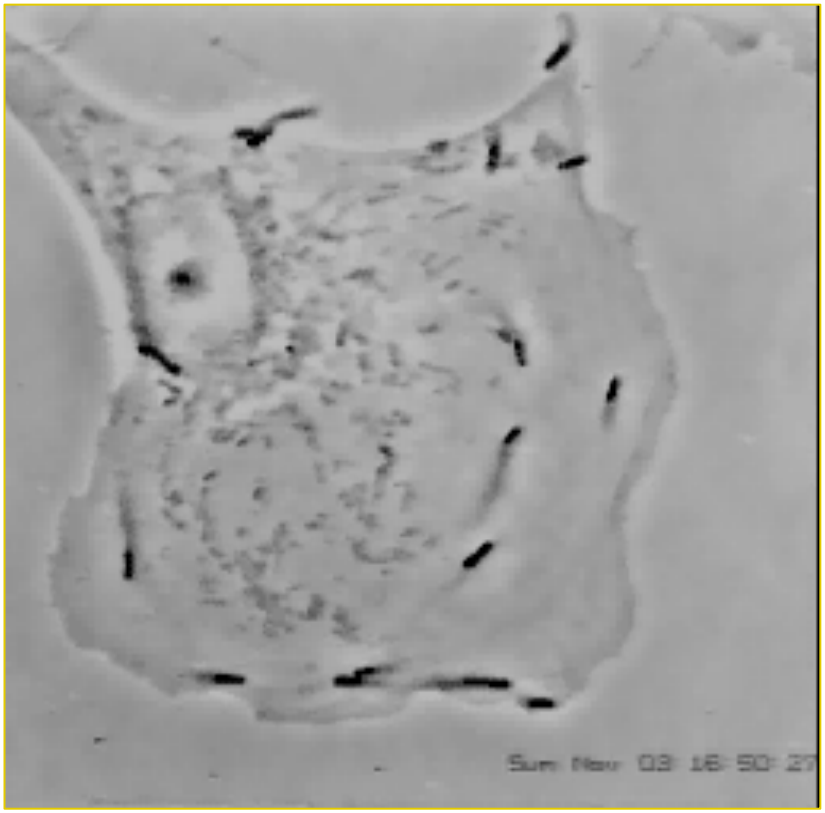




ActA





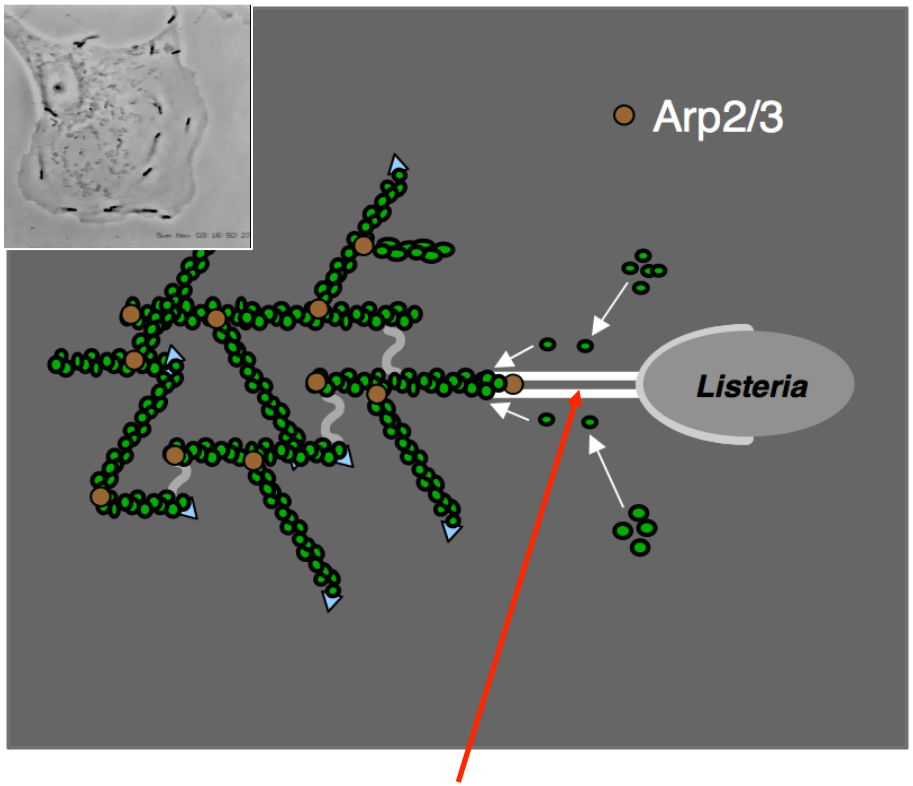


ActA has been a tool for the discovery of the role of the **Arp2/3 complex**

Kocks et al.,Cell, 1992

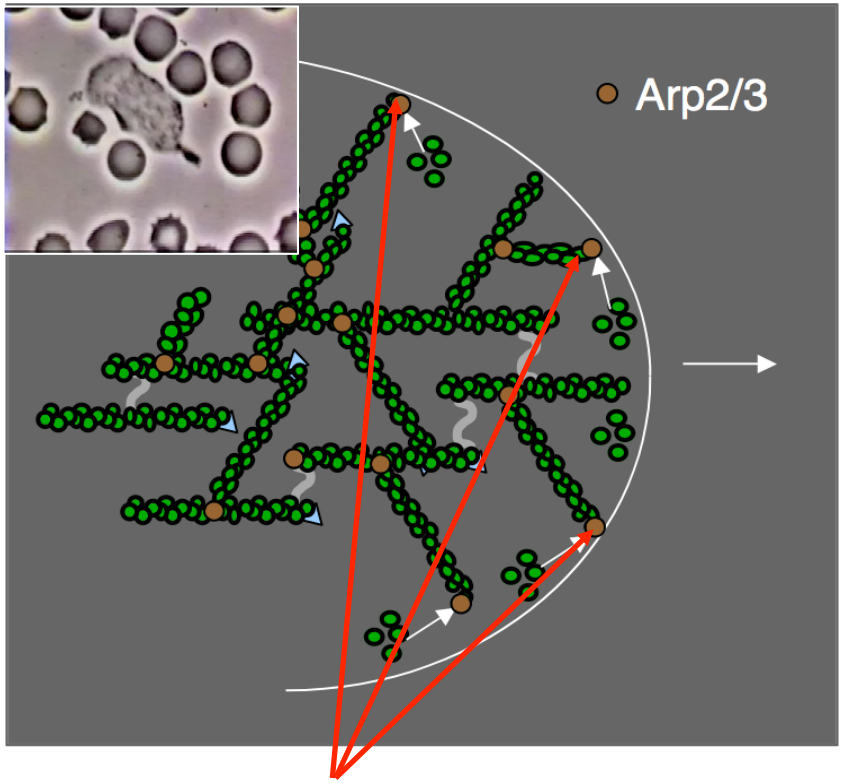
ActA or WASP recruit the **Arp2/3 complex**

Locomoting *Listeria*



ActA

Leading edge of a moving cell



Scar/ WASP family proteins

Actin-based motility

Listeria is not the only bacterium able to move from cell to cell.

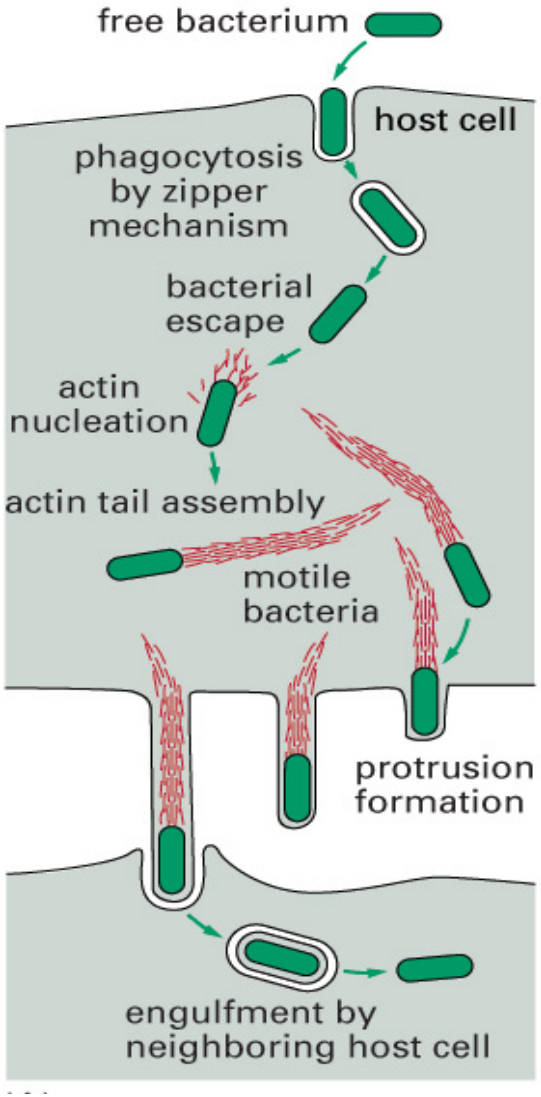
<u>Other bacteria able to polymerize actin include</u>

Shigella flexneri : IcsA/VirG + N-WASP+ Arp2/3

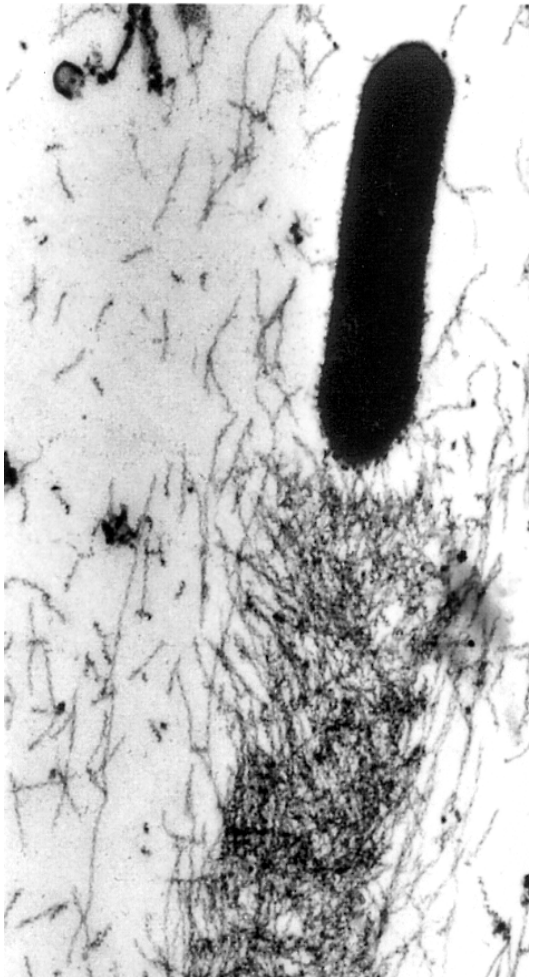
Rickettsia conorii : RickA + Arp2/3, Sca2

Burkholderia : BtBimA, BpBimA, BmBimA

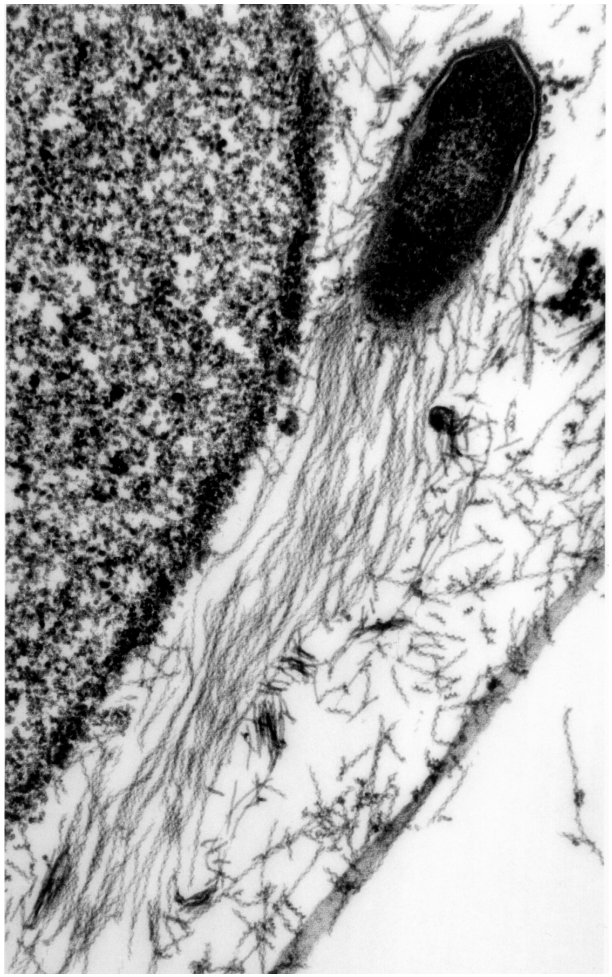
Mycobacterium marinum : a new protein



Two types of comet tails

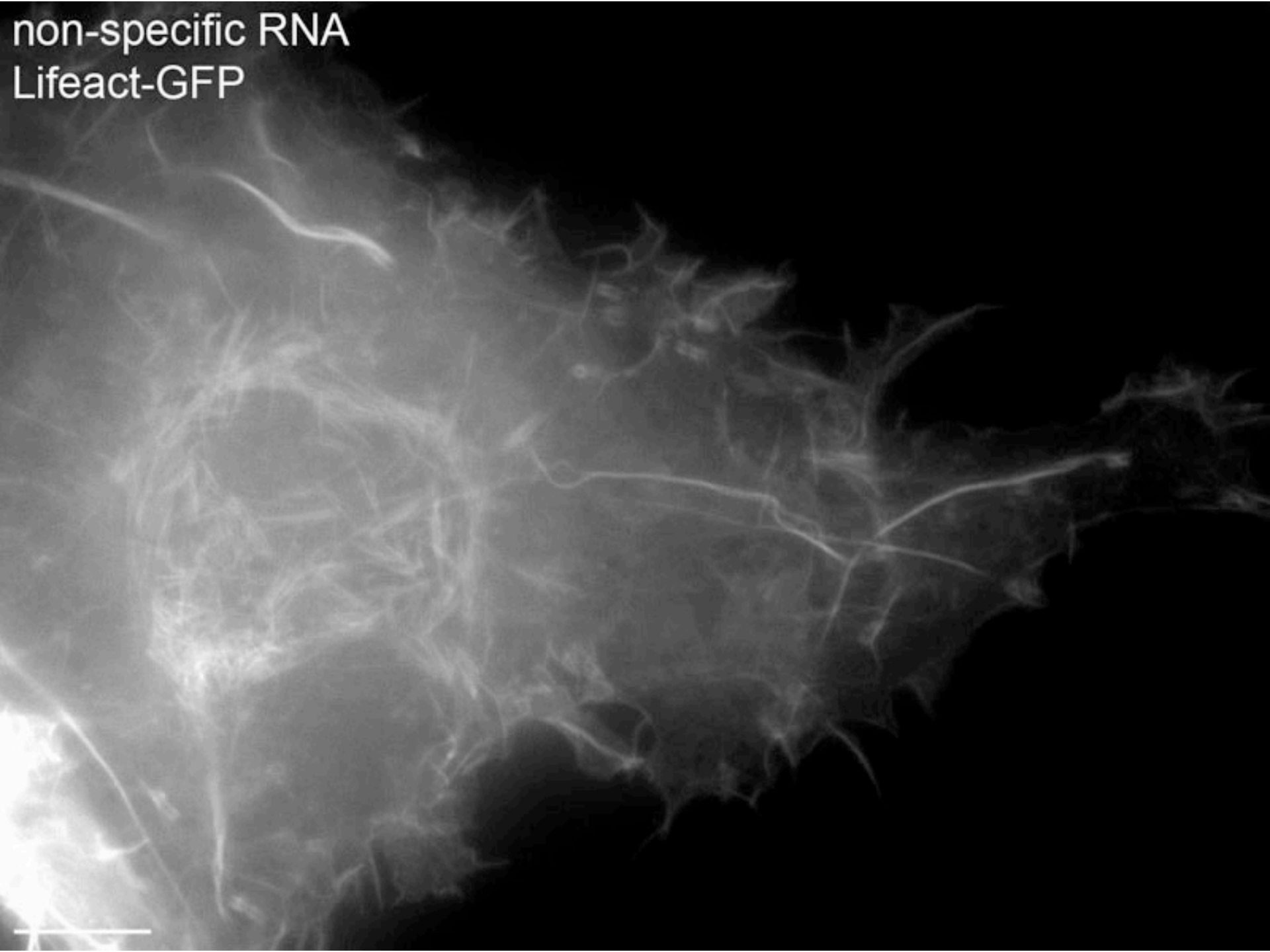


L. monocytogenes: ActA



R. conorii: RickA

Gouin et al. Nature 2004


non-specific RNA
Lifeact-GFP

Actin-based motility

Listeria is not the only bacterium
able to move from cell to cell.

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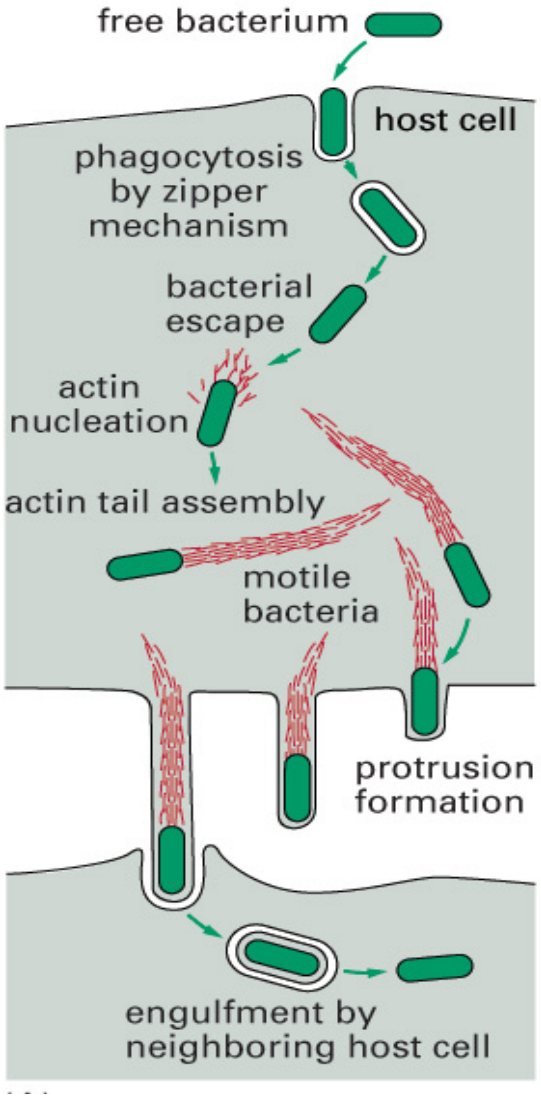
Mycobacterium marinum : a new protein



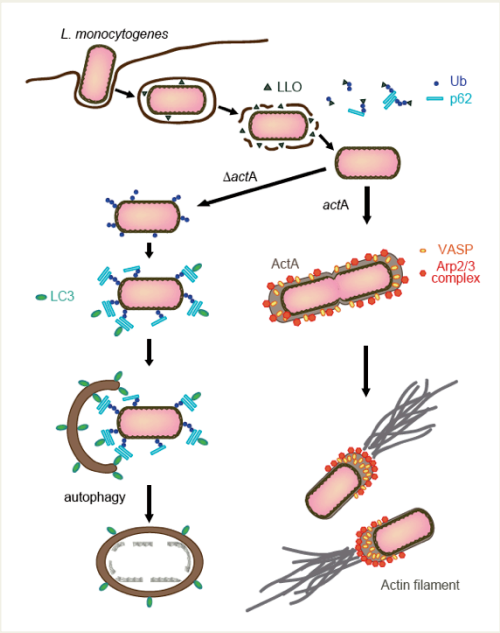
ActA protects the bacterium from autophagy

nature cell biology

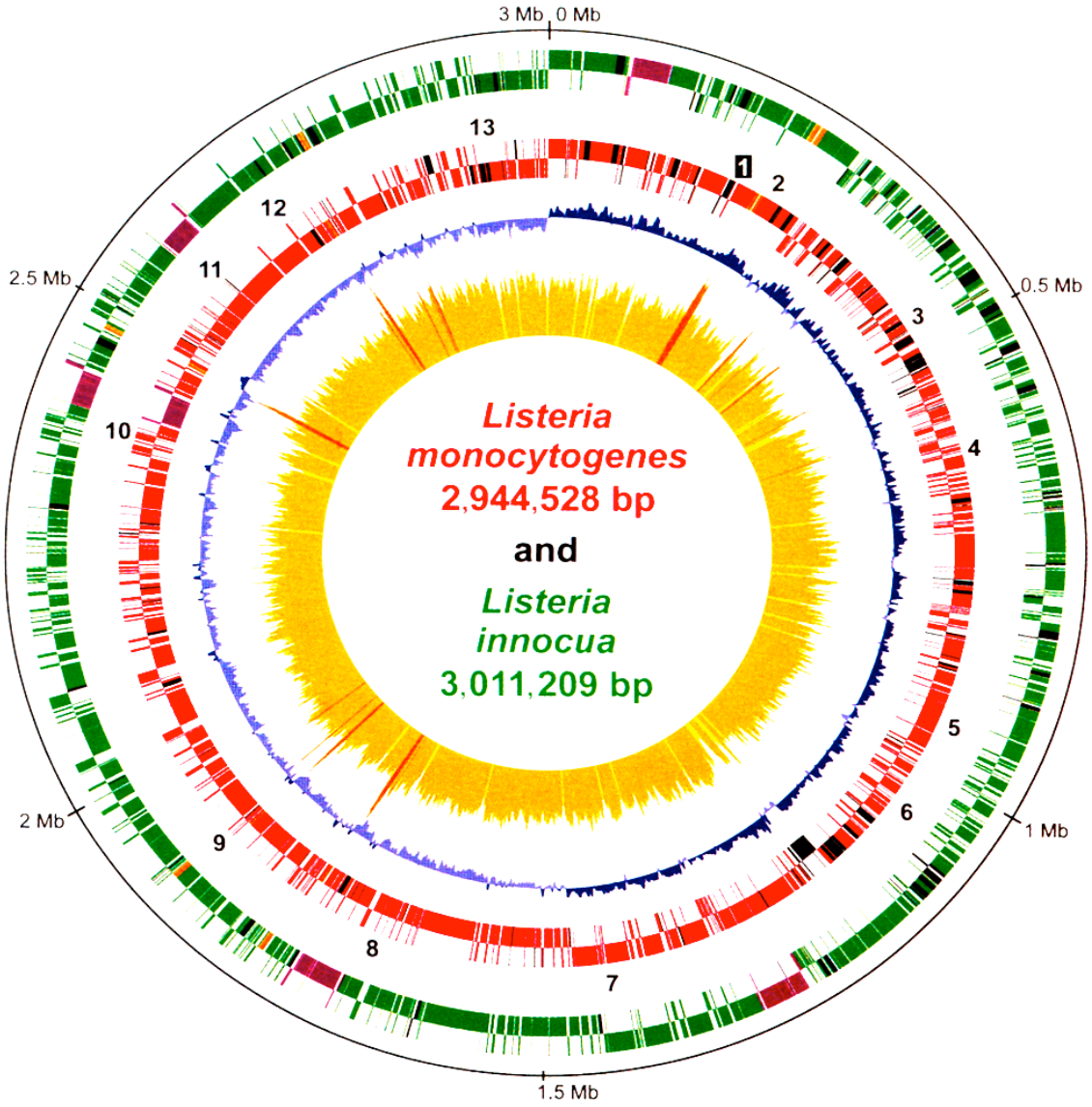
Listeria monocytogenes ActA-mediated escape from autophagic recognition

Yuko Yoshikawa[1], Michinaga Ogawa[1], Torsten Hain[2], Mitsutaka Yoshida[3], Makoto Fukumatsu[1], Minsoo Kim[4], Hitomi Mimuro[1], Ichiro Nakagawa[4], Toru Yanagawa[5], Tetsuro Ishii[5], Akira Kakizuka[6], Elizabeth Sztul[7], Trinad Chakraborty[2] and Chihiro Sasakawa[1,4,8,9]

published online 13 September 2009



Post-genomic studies



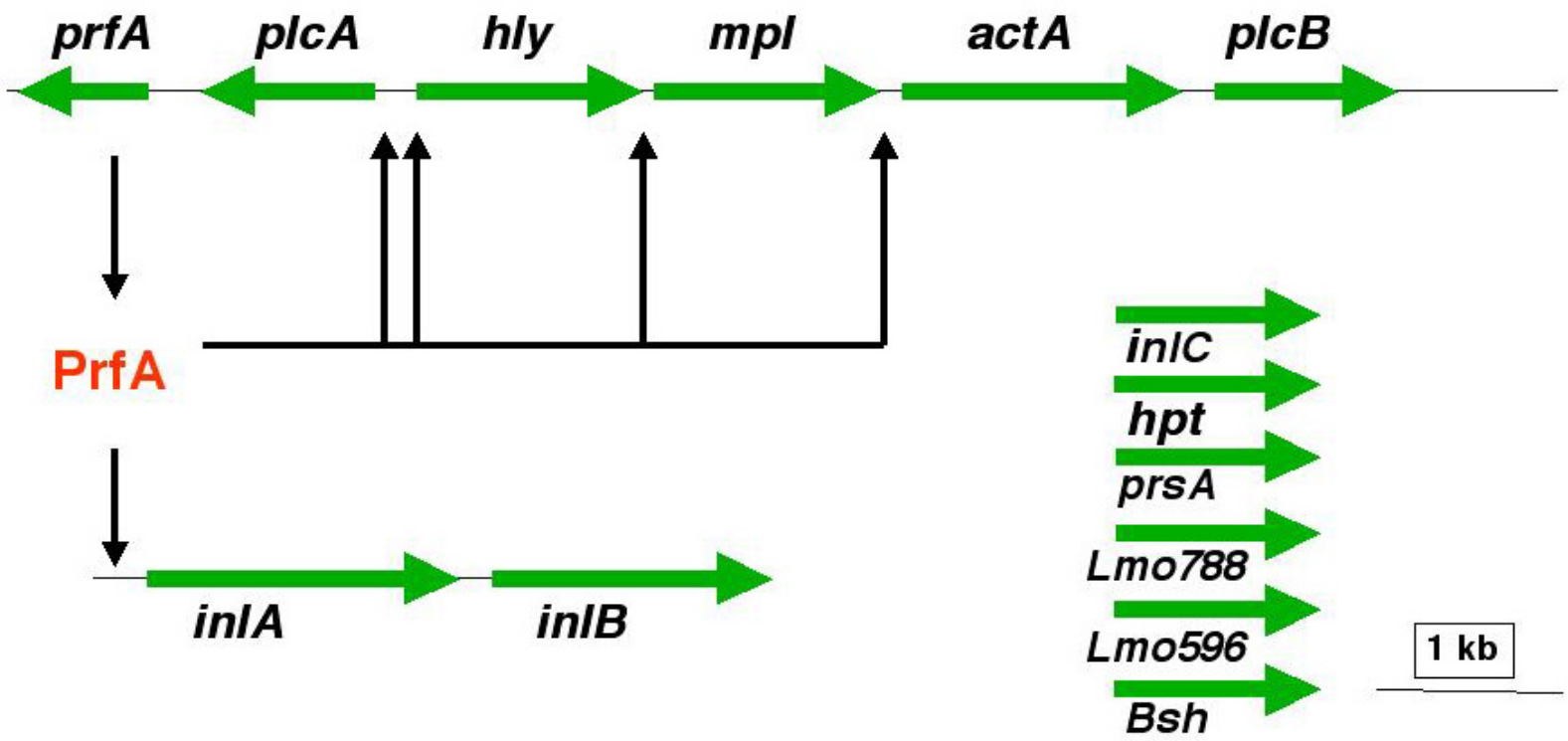
Biodiversity

Virulence

Global gene expression

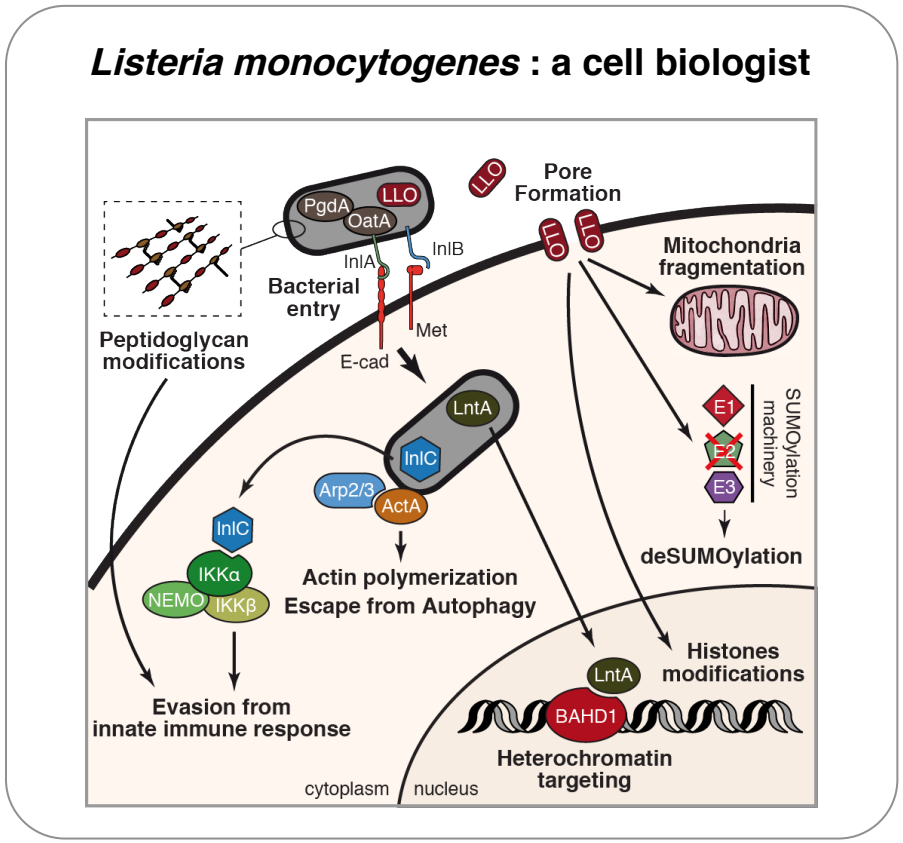
Glaser *et al.*, Science 2001

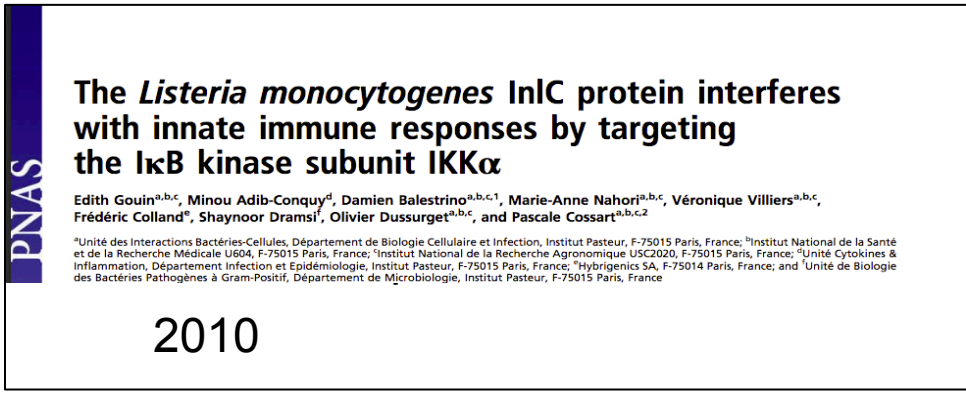
The PrfA regulon

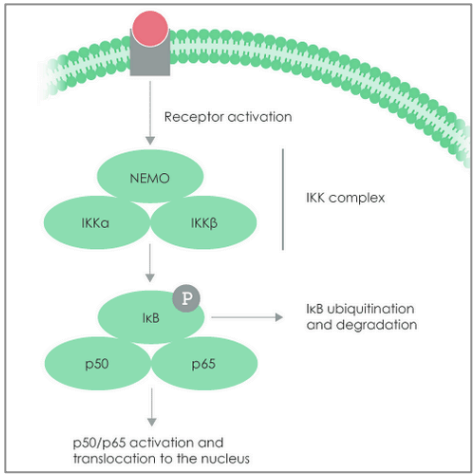


Milohanic *et al*. Mol. Microbiol 2003

InlC : a secreted protein that interferes with the NF-kB pathway

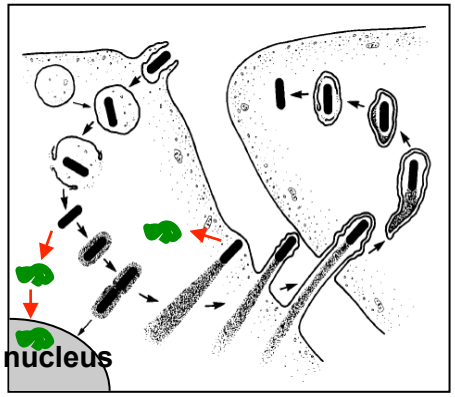




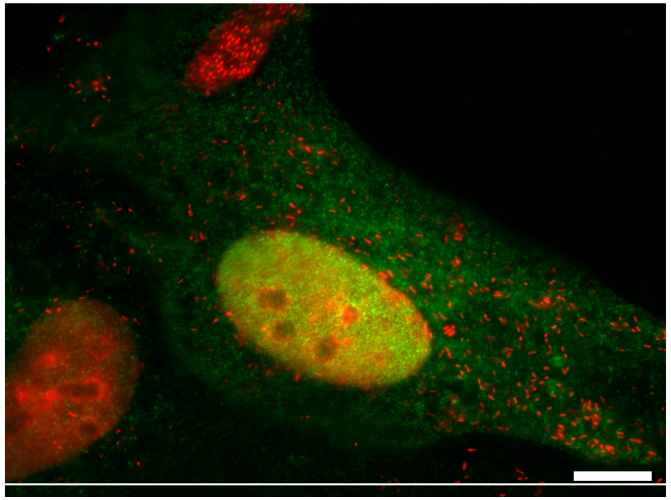


LntA : a secreted protein which goes to the nucleus

LntA= *Listeria* nuclear targeted protein A

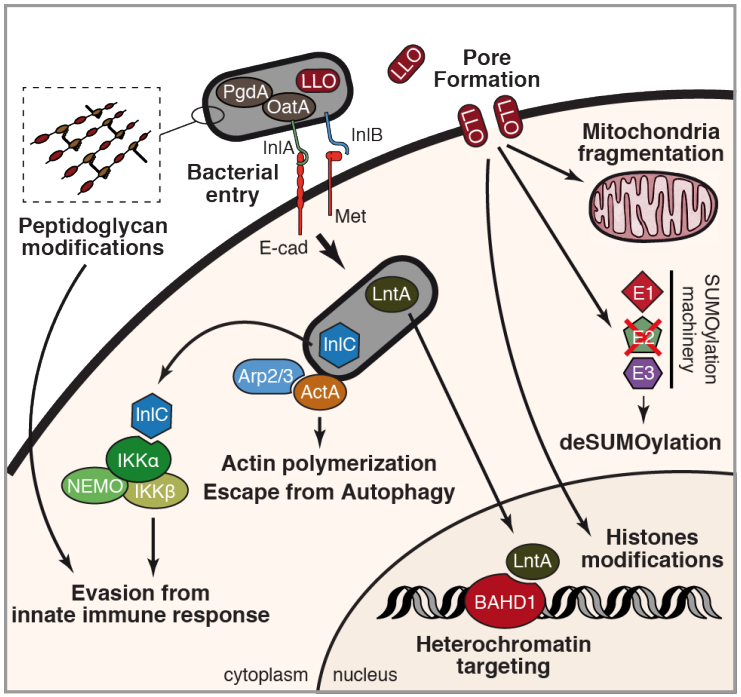


LntA + DAPI

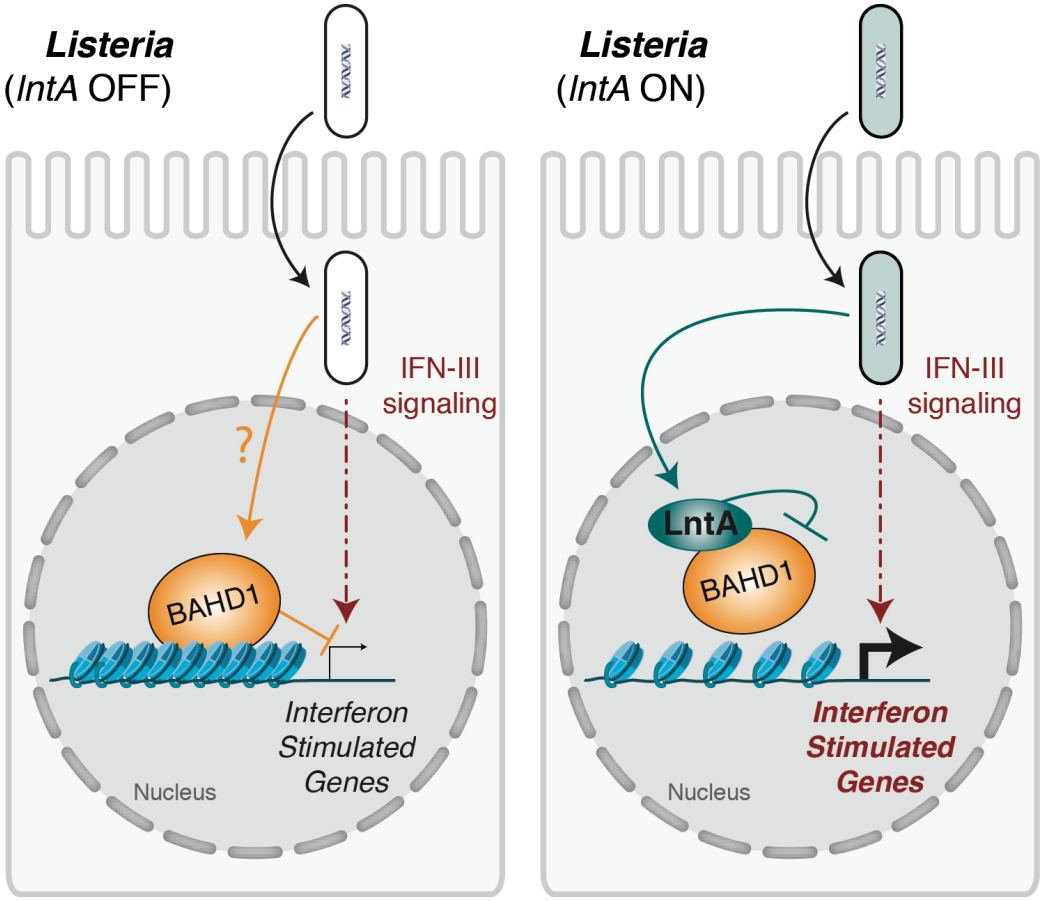


human fibroblasts infected for 24h with *lntA*-expressing *Listeria*

***Listeria monocytogenes* : a cell biologist**

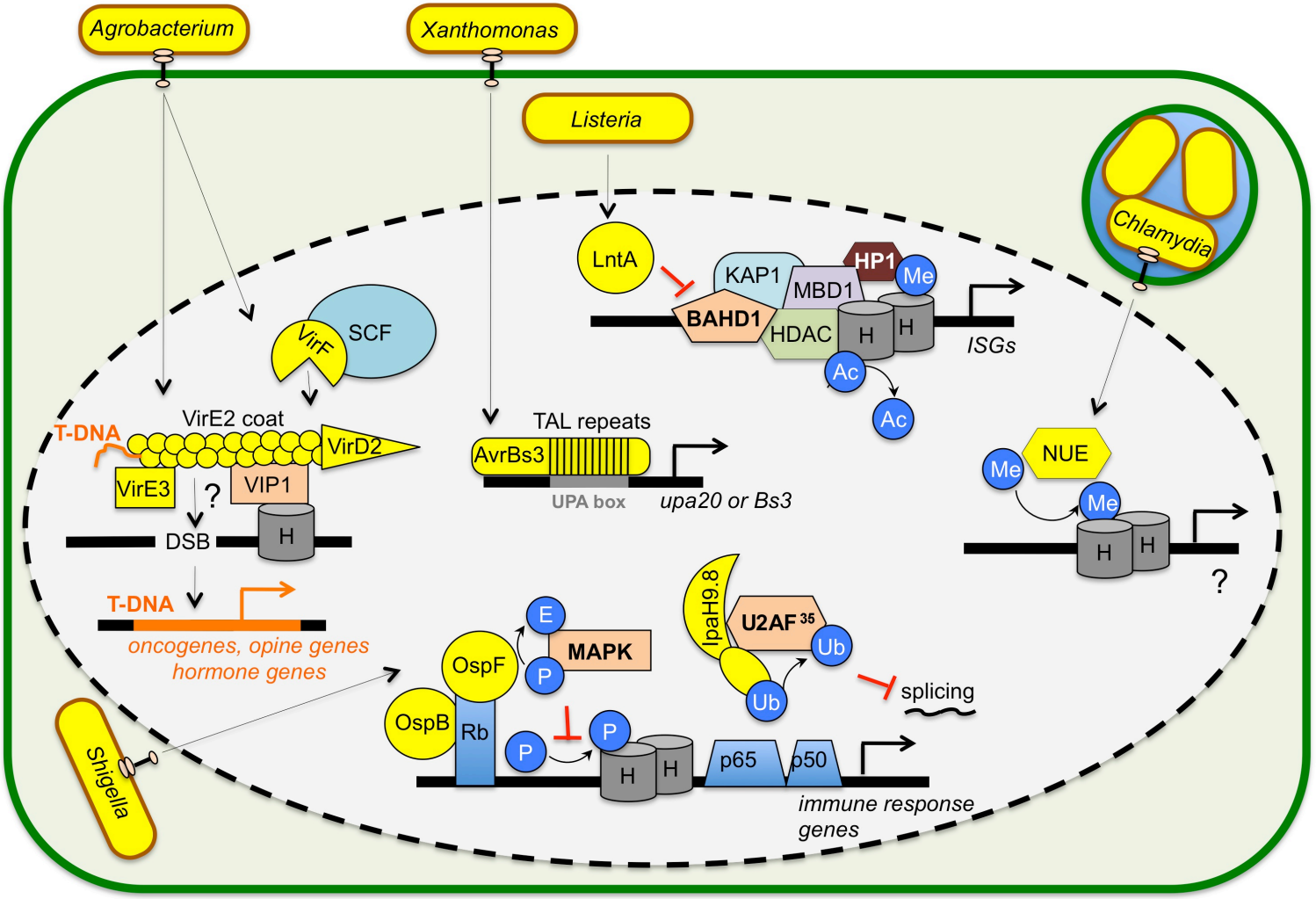


LntA and **BAHD1** control the interferon type III response



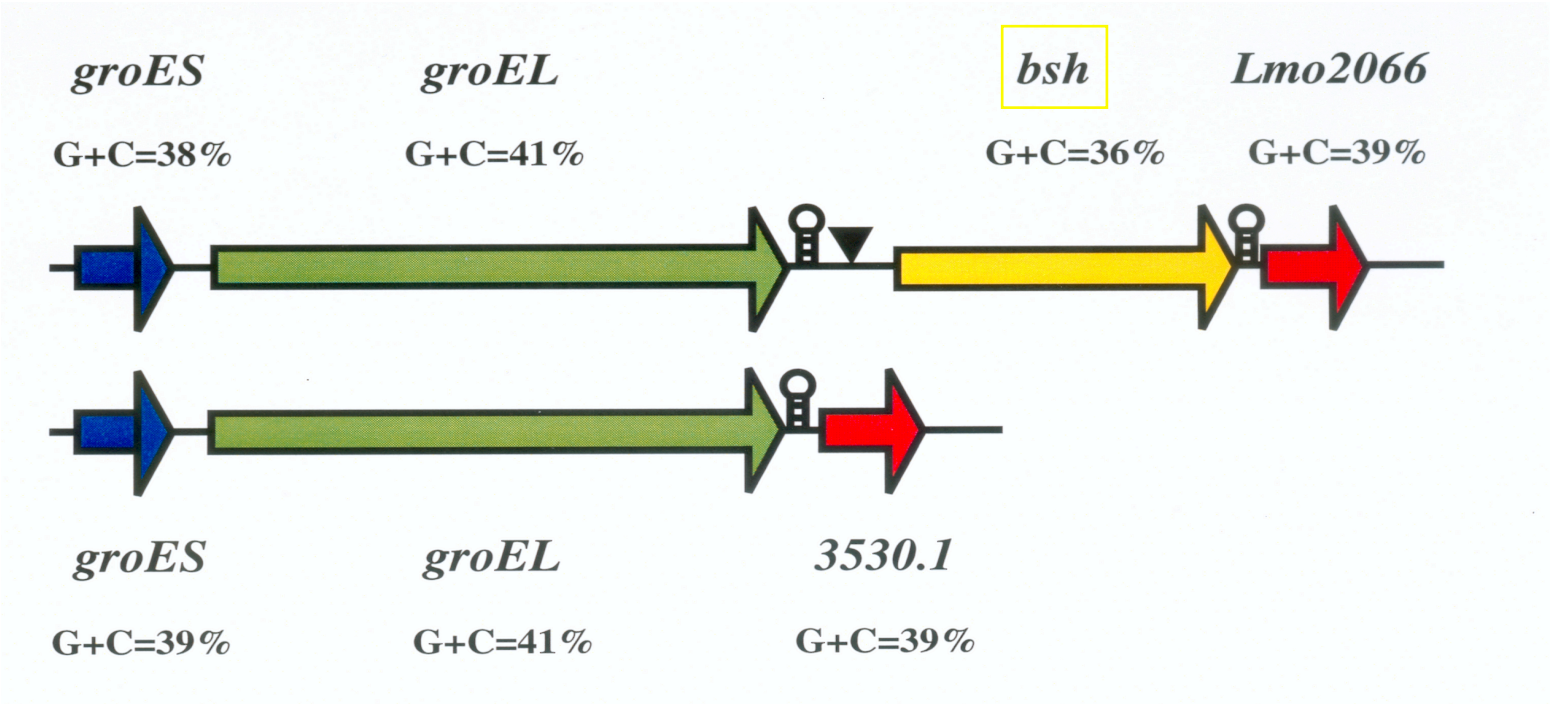
Lebreton et al., Science 2011; Lebreton et al., mBio 2014

LntA defines a novel family of bacterial proteins: **the nucleomodulins**



Bierne and Cossart, 2012. Cell microbiol.

bsh *: a* gene that encodes BSH, a bile salt hydrolase

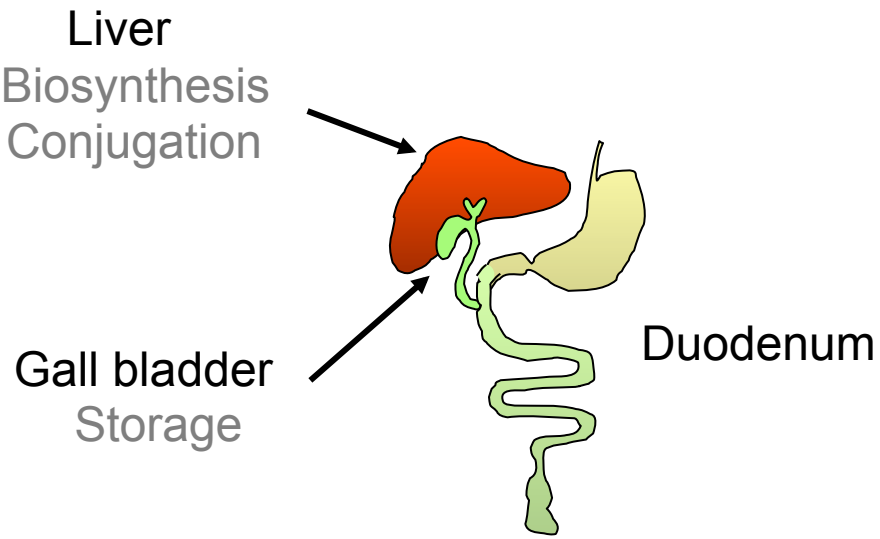


Dussurget et al. 2002 Mol Microbiol

Bile and bile salts

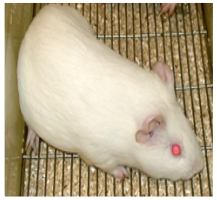
Bile is a complex liquid made of cholesterol-derived compounds

Role: **Emulsification** of dietary lipids, **antimicrobial** agent

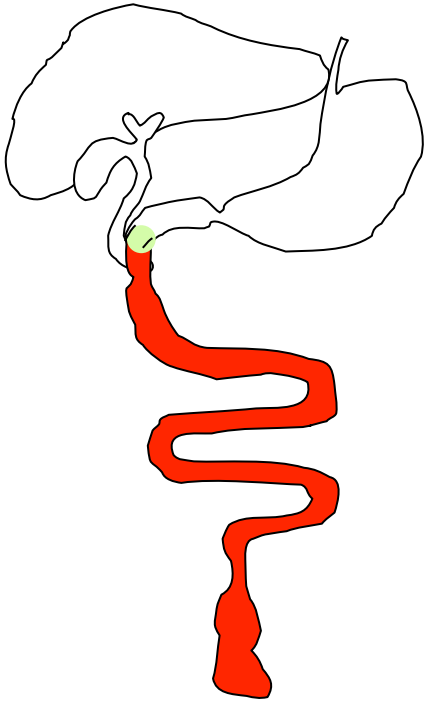


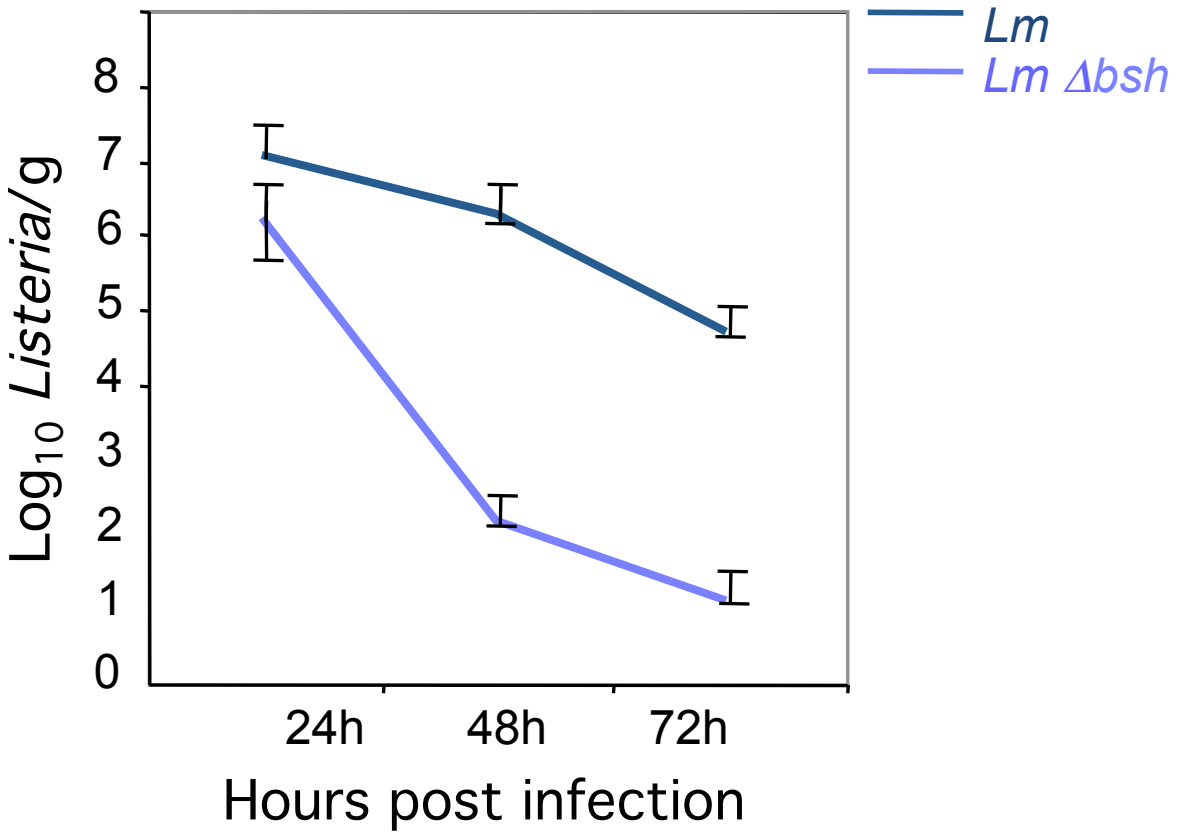
Intestinal commensals have evolved tricks to resist to the bactericidal action of bile salts

BSH : an enzyme critical for persistence in the intestine



Oral infection of guinea pigs





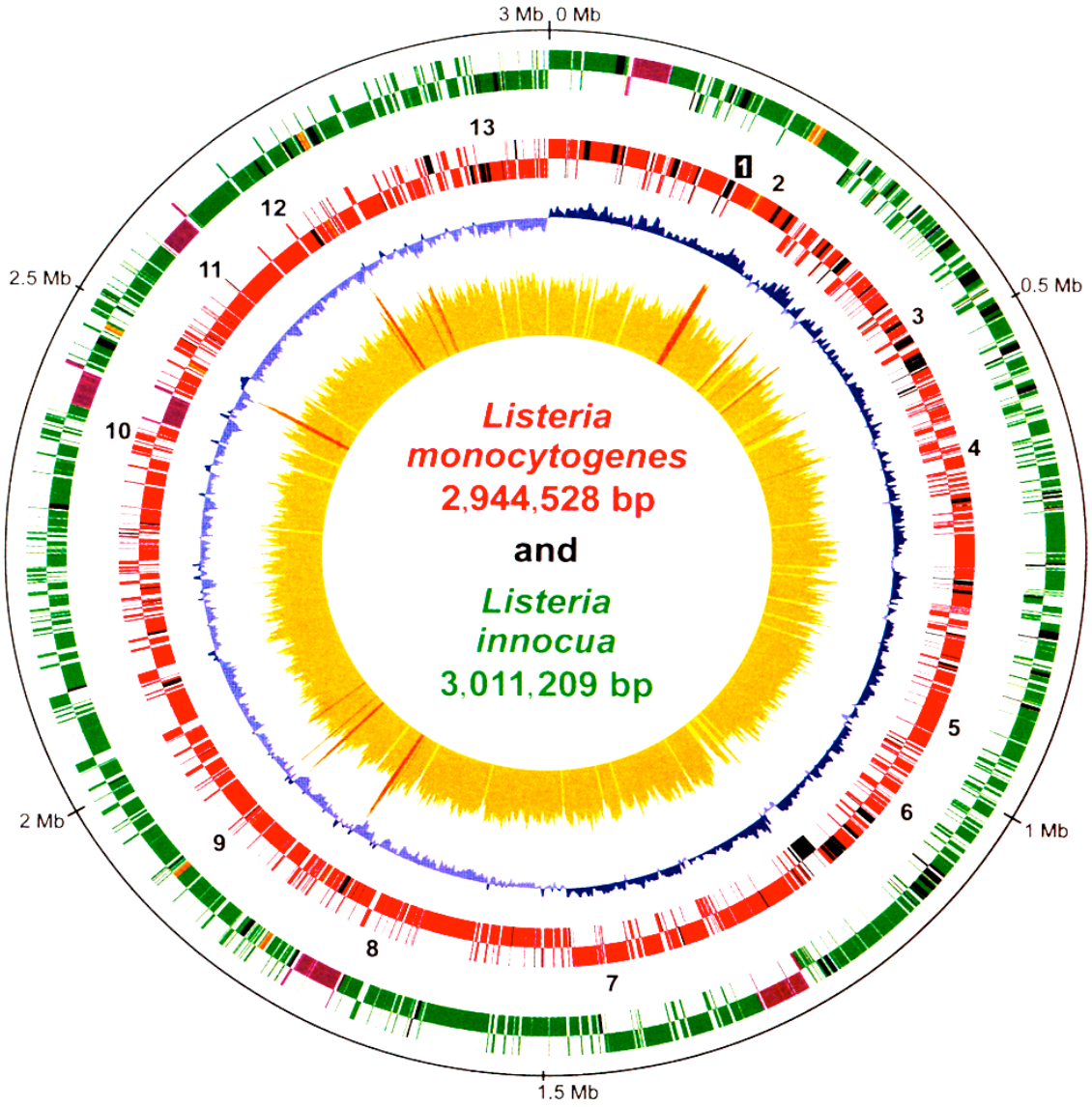
Dussurget et al. 2002 Mol Microbiol

BSH: a novel type of virulence factor

Similar to commensals,
pathogenic *Listeria* counteract the antibacterial action
of bile salts by the action of bile salt hydrolase (BSH)

This allows persistence in the intestine and
is critical for virulence

Post-genomic studies



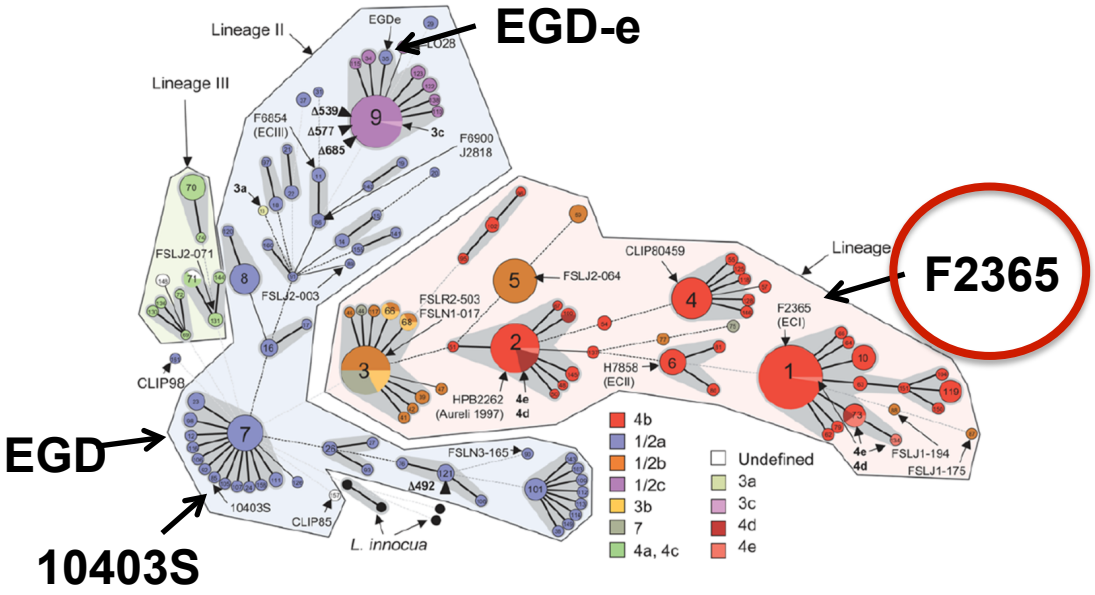
Biodiversity

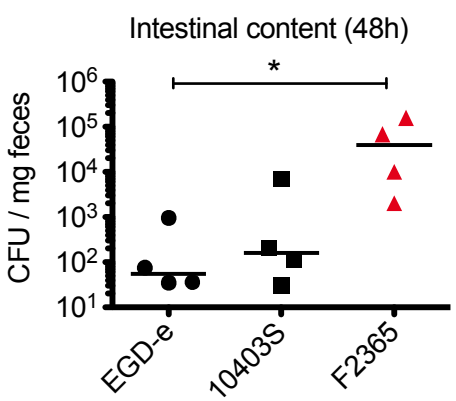
Virulence

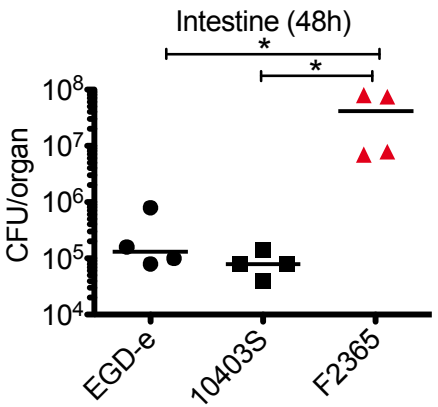
Global gene expression

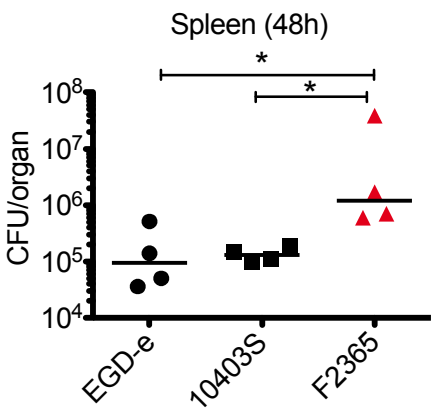
Glaser *et al.*, Science 2001

Lineage I epidemic strain F2365 is more virulent upon oral infection









Quereda et al., PNAS, 2016

Listeriolysin S (LLS) is present in strains of *Listeria* from epidemics

OPEN ACCESS Freely available online

PLoS PATHOGENS

Listeriolysin S, a Novel Peptide Haemolysin Associated with a Subset of Lineage I *Listeria monocytogenes*

Paul D. Cotter[1]*, Lorraine A. Draper[1], Elaine M. Lawton[1], Karen M. Daly[1], David S. Groeger[2], Pat G. Casey[1,3], R. Paul Ross[3,4], Colin Hill[1,3]*

1 Department of Microbiology, University College Cork, Cork, Ireland, **2** Alimentary Health Ltd., Cork, Ireland, **3** Alimentary Pharmabiotic Centre, University College Cork, Cork, Ireland, **4** Moorepark Food Research Centre, Teagasc, Moorepark, Fermoy, Cork, Ireland

Editor: Pascale Cossart, Institut Pasteur, France

Received March 18, 2008; **Accepted** August 7, 2008; **Published** September 12, 2008

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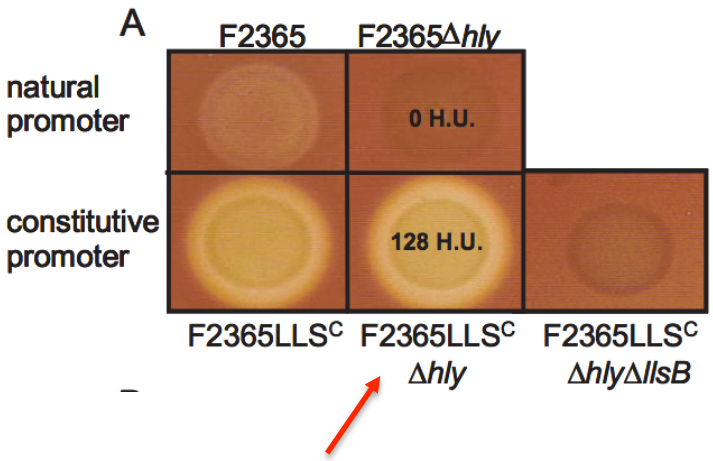
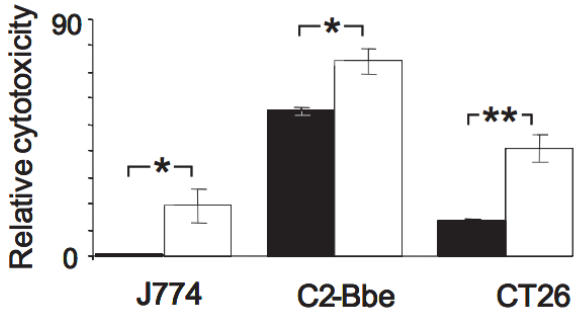
Competing Interests: The authors have declared that no competing interests exist.

Hemolytic activity

(not expressed in broth media with the natural promoter)



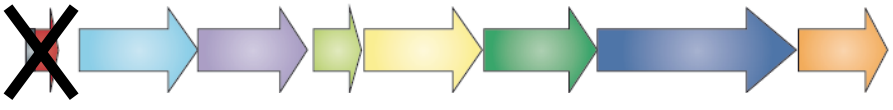
in vitro



LLS is a cytotoxin

LLS is the first reported *Listeria* bacteriocin

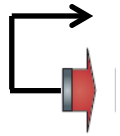
Listeriolysin S (*L. monocytogenes* F2365)



Toxin | Transporter | PTM enzymes | Immunity protein

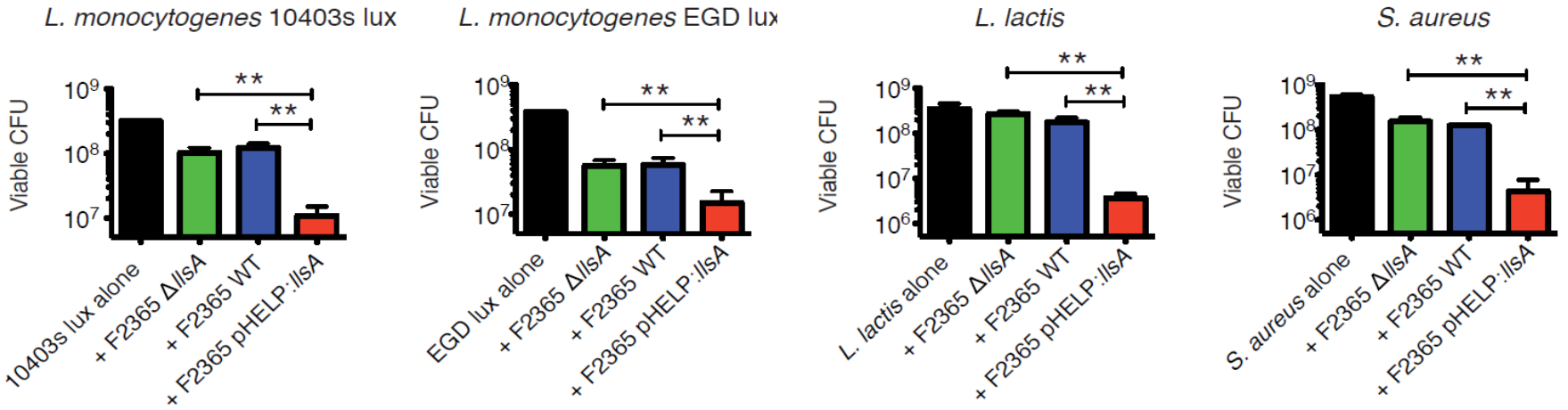
overexpression

pHELP



Toxin

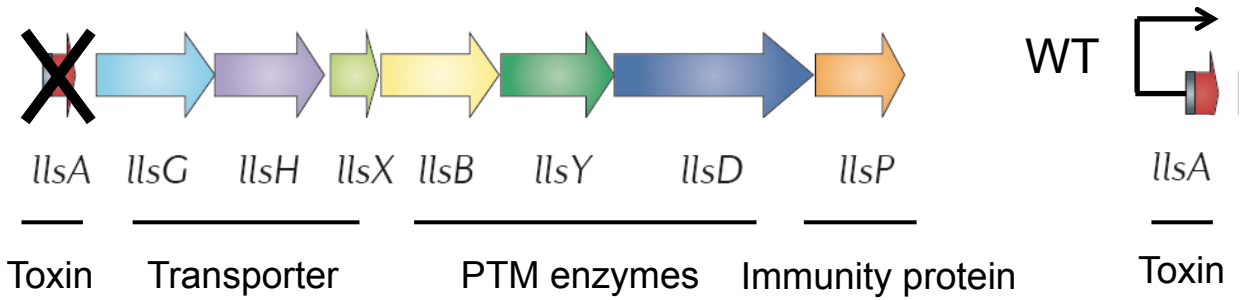
In vitro

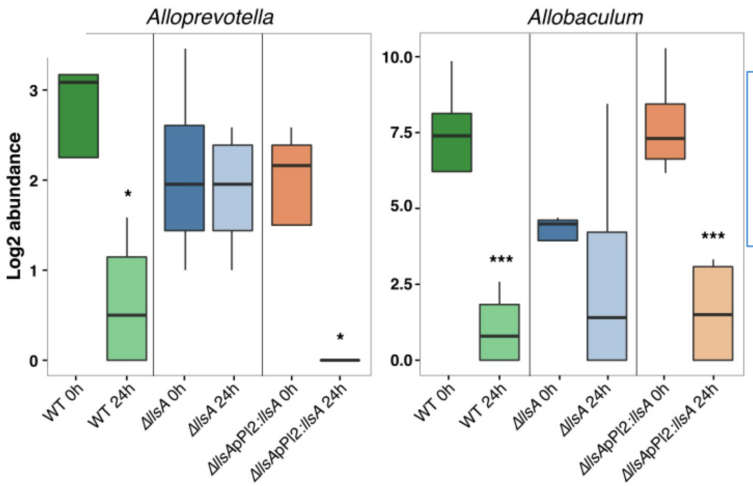


Quereda et al., PNAS, 2016

LLS acts on the intestinal microbiota

Listeriolysin S (*L. monocytogenes* F2365)



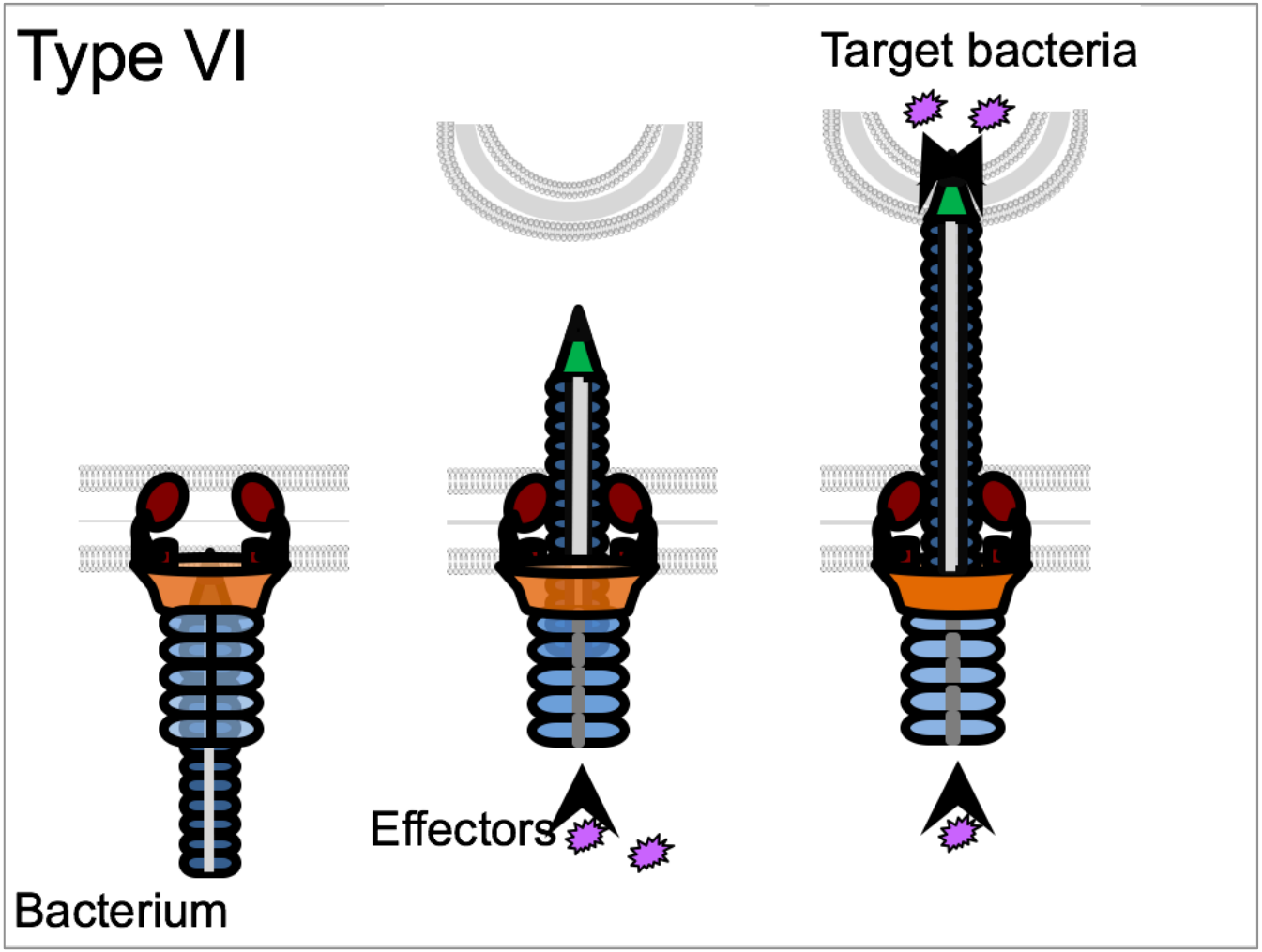


- ***Alloprevotella*** **produces acetic acid which inhibits** ***Listeria*** **growth**

- ***Allobaculum*** **produces butyric acid which inhibits virulence factor production in** ***Listeria***

Quereda et al., PNAS, 2016
Quereda et al., mBio, 2017

Bacteriocins and type VI secretion systems are used by bacteria to target other bacteria in particular in the gut



- **Genetic approches** : InlA, LLO, ActA

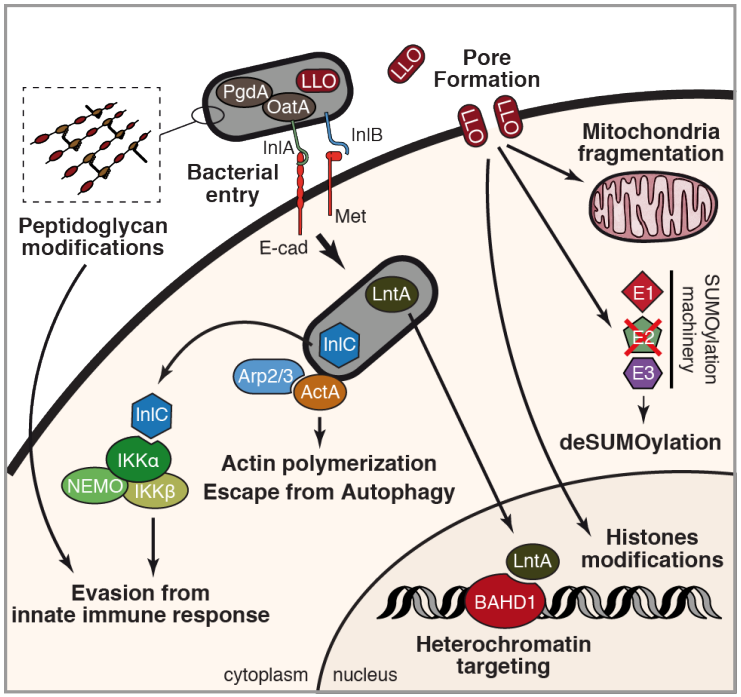
- **Post genomic approches**
  - Surface and secreted virulence factors : InlC, LntA, BSH
  - Small RNAs and riboregulators : *not discussed*
  - Small proteins and stressosome : *not discussed*
  - Bacteriocin : more to come

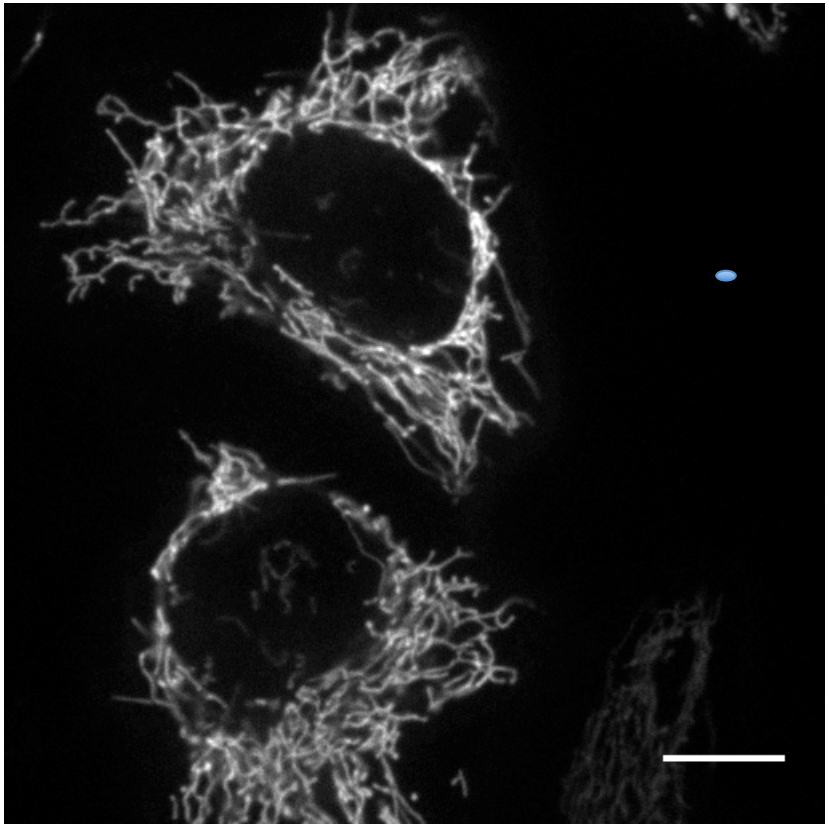
- **Hypothesis-driven approahes**

  Histone modifications
  Post-translational modifications
  Mitochondrial dynamics

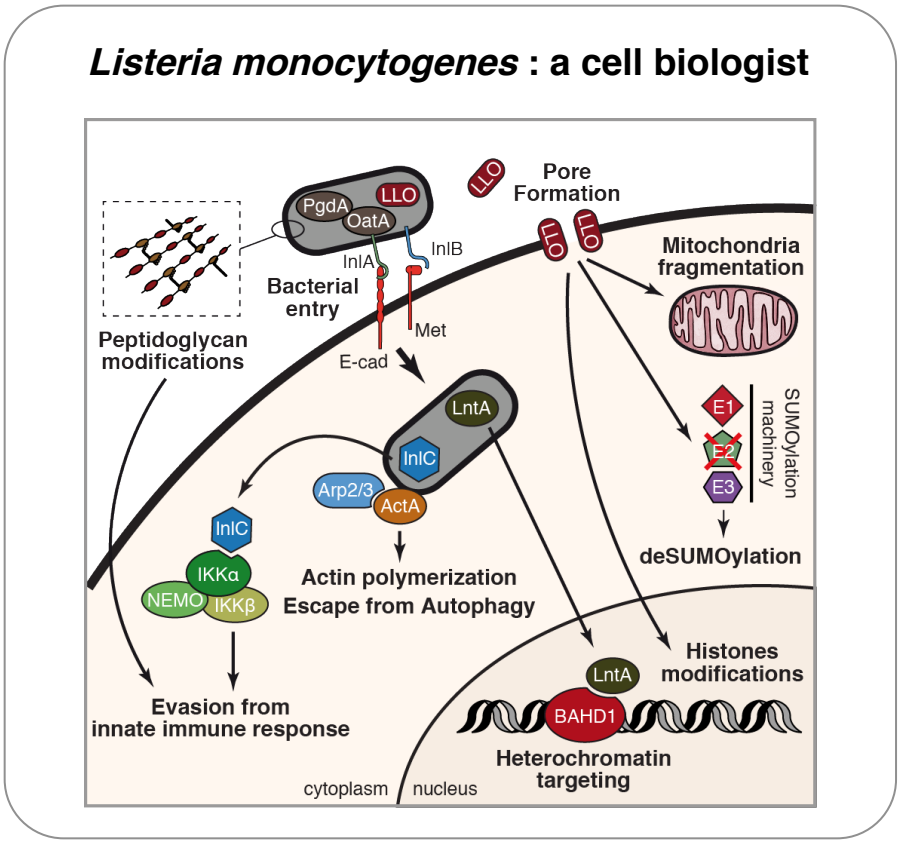
Listeria infection and mitochondrial dynamics

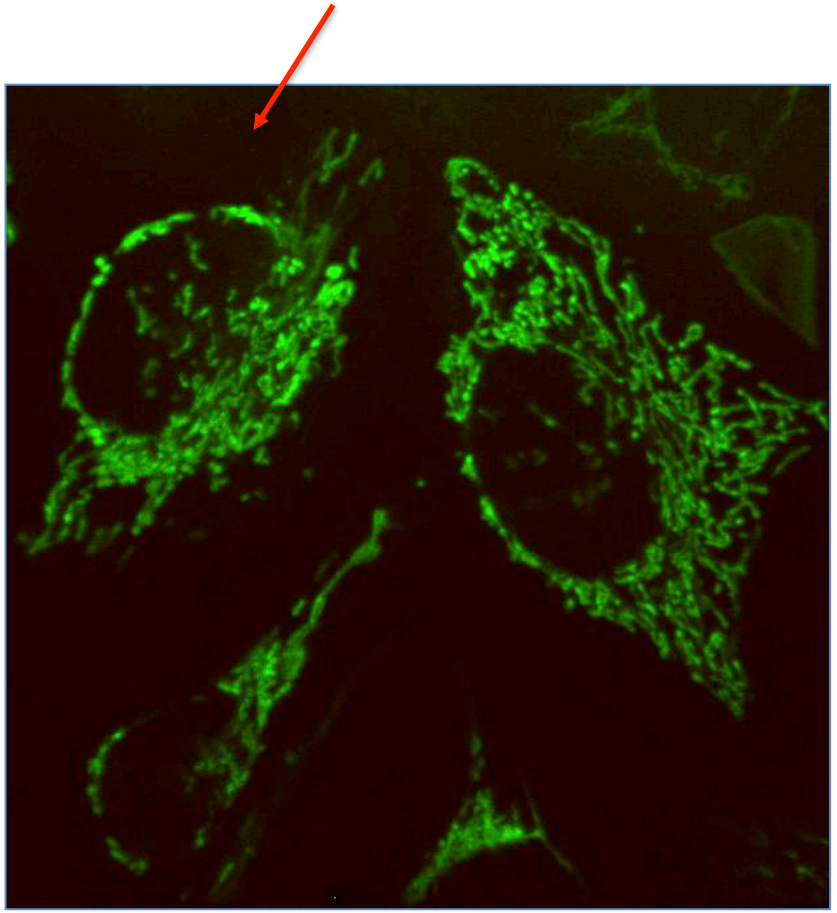
Listeria monocytogenes **: a cell biologist**





Listeria infection causes mitochondrial fragmentation





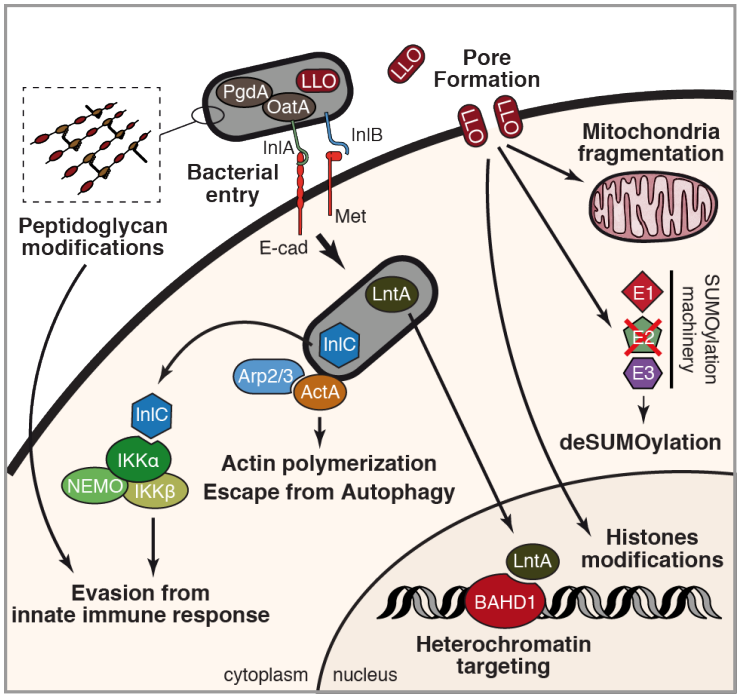
This fragmentation is required for an efficient infection

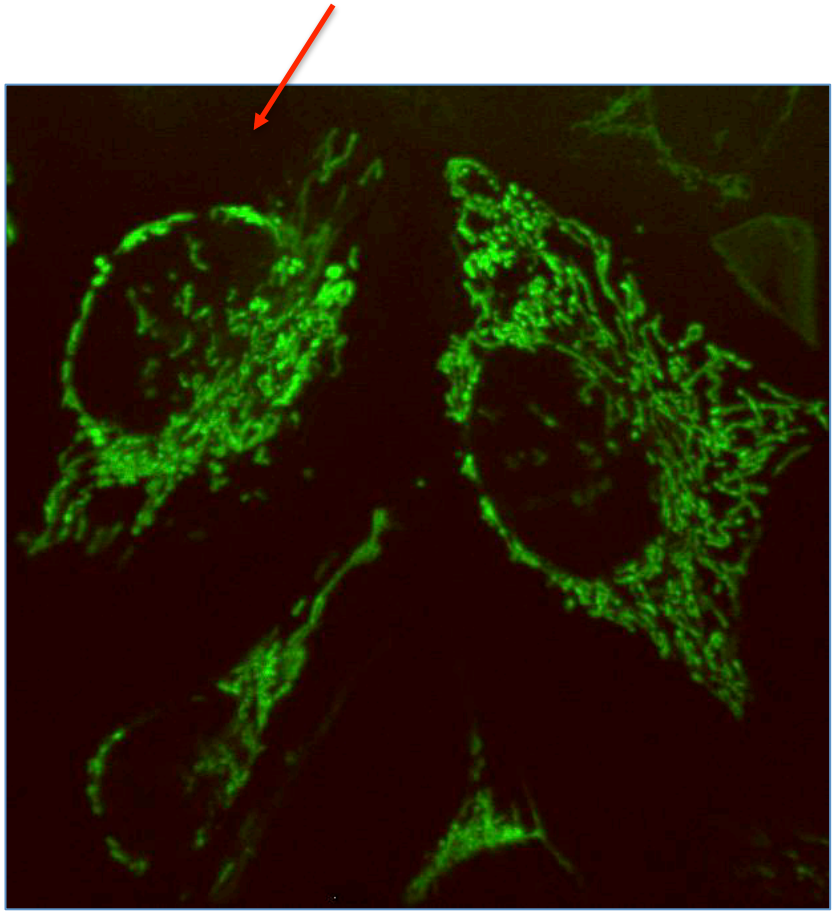
Stavru et al., PNAS, 2011; 2013

Pagliuso et al., Embo rep. 2017

Listeria infection causes mitochondrial fragmentation

Listeria monocytogenes **: a cell biologist**





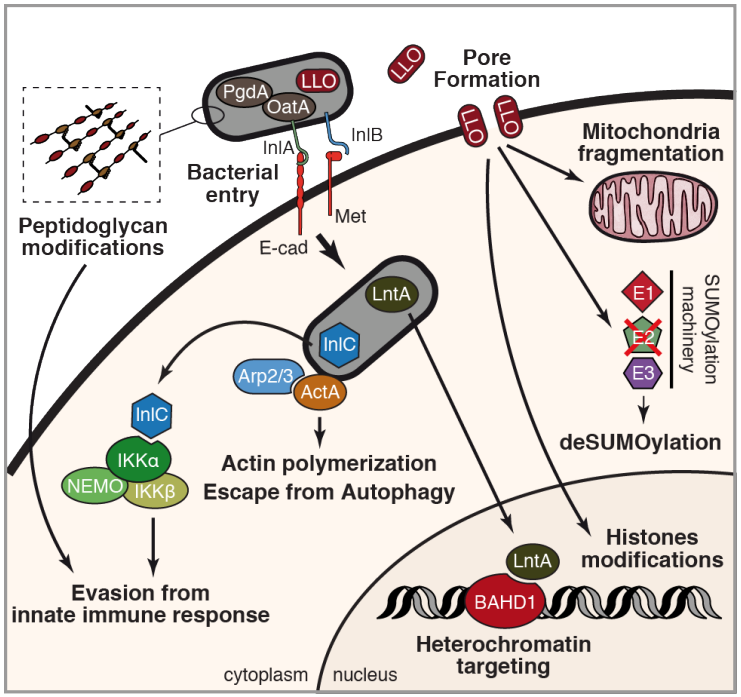
Listeria **mediates a Drp1-independent fragmentation critical for infection**

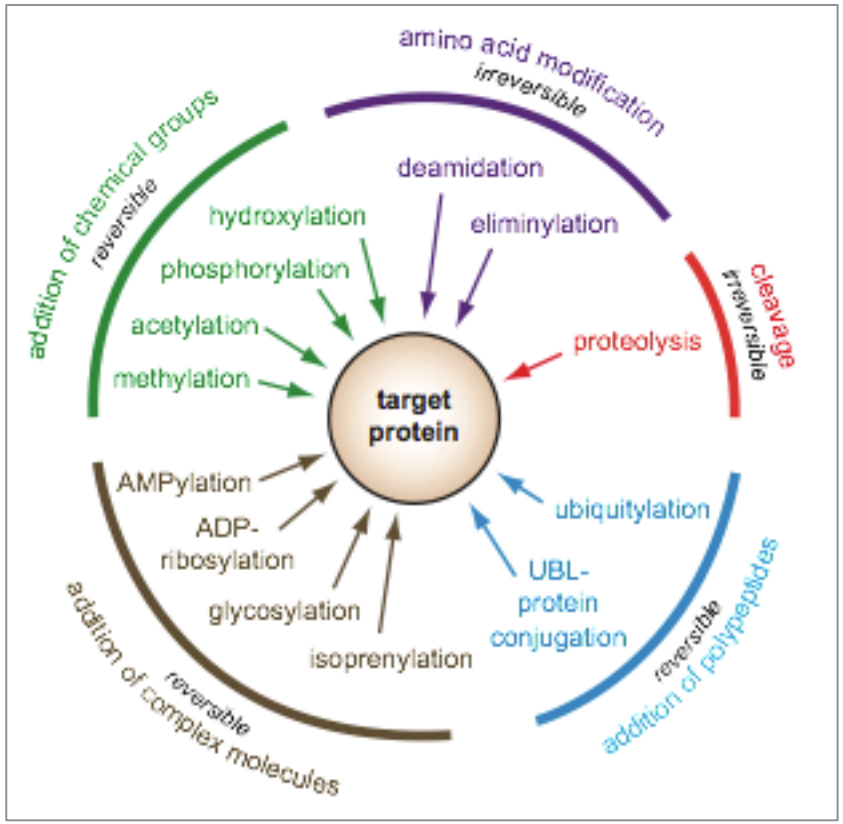
Stavru et al., PNAS, 2011; 2013

Pagliuso et al., Embo rep. 2017

Listeria infection and post-translational modifications

Listeria monocytogenes **: a cell biologist**

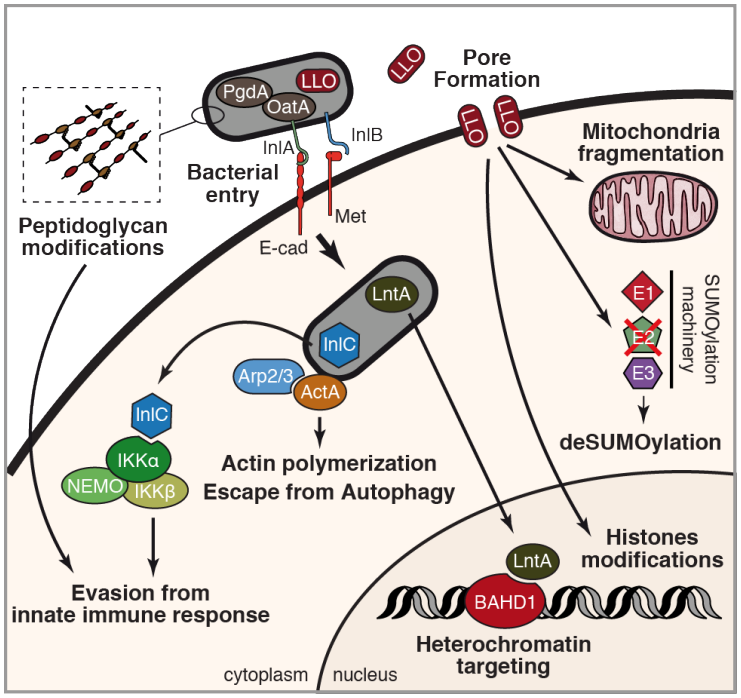


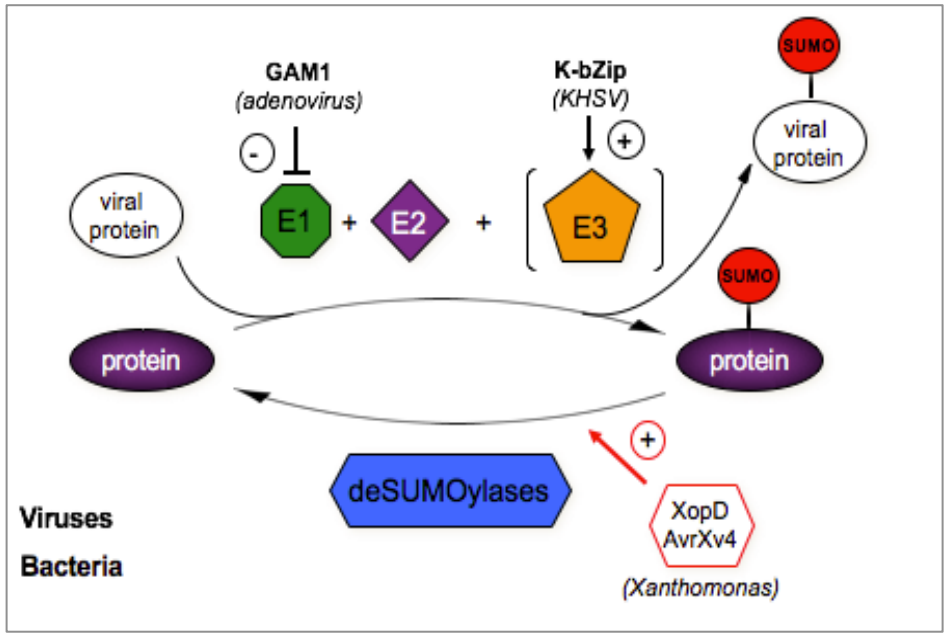


Modifications of protein activities or interactions : locally, quickly , specifically

Listeria infection and the SUMO pathway

***Listeria monocytogenes* : a cell biologist**

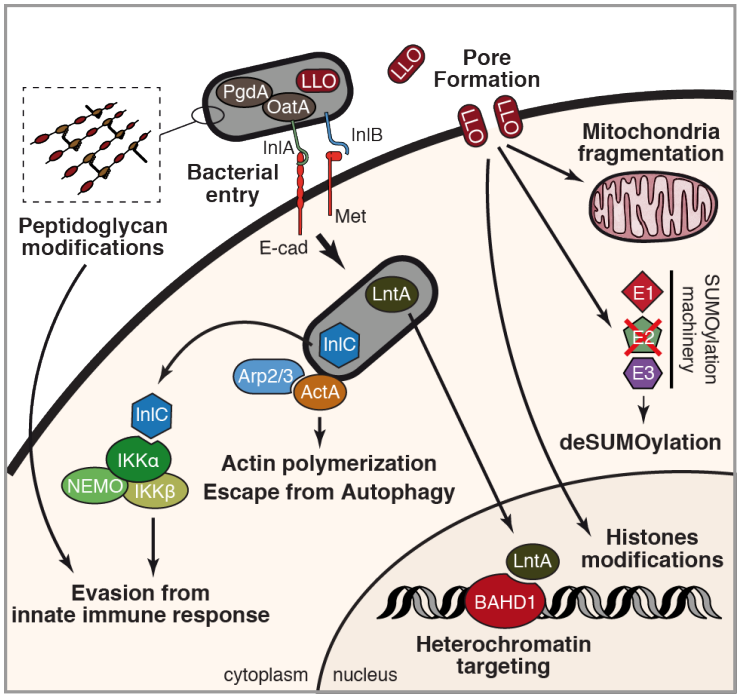


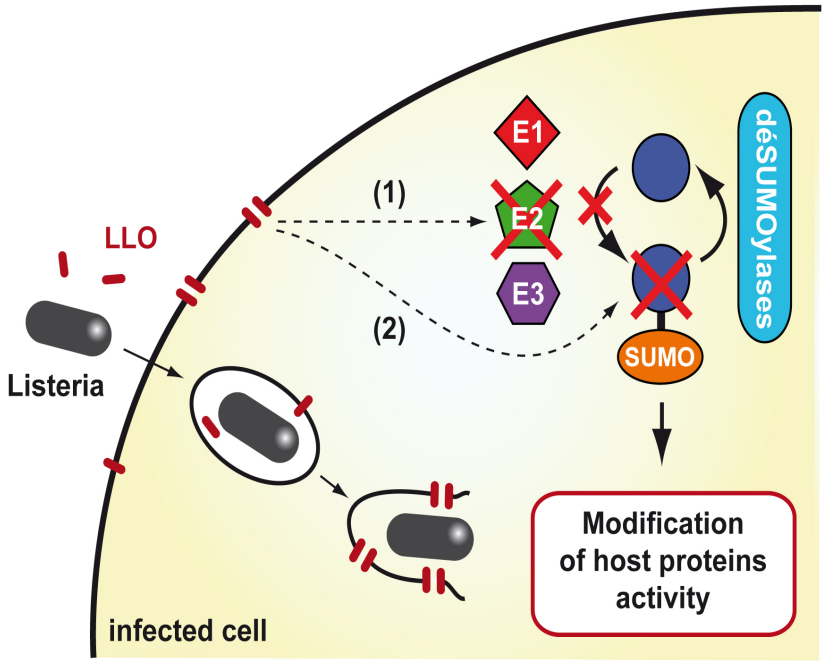


***Listeria* infection induces the degradation of UBC9 via LLO**

Listeria infection and the SUMO pathway

Listeria monocytogenes **: a cell biologist**

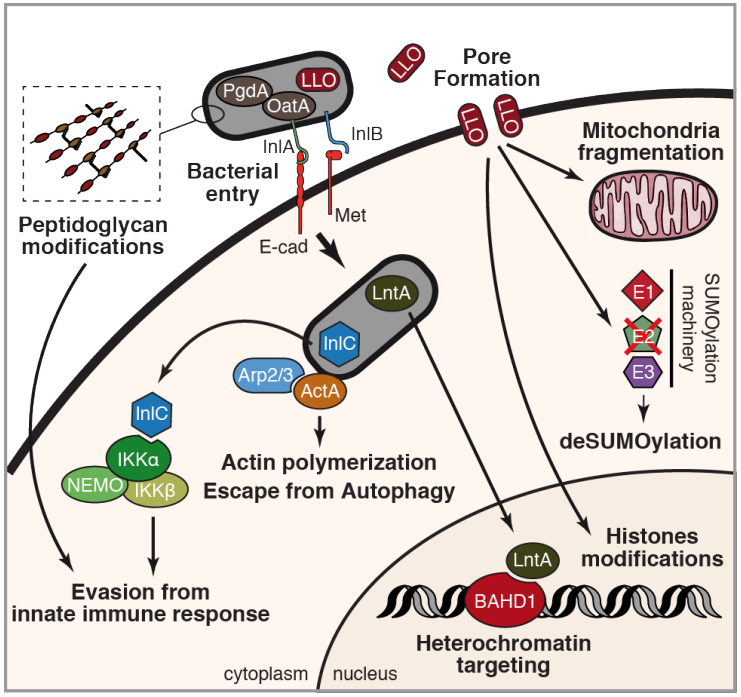




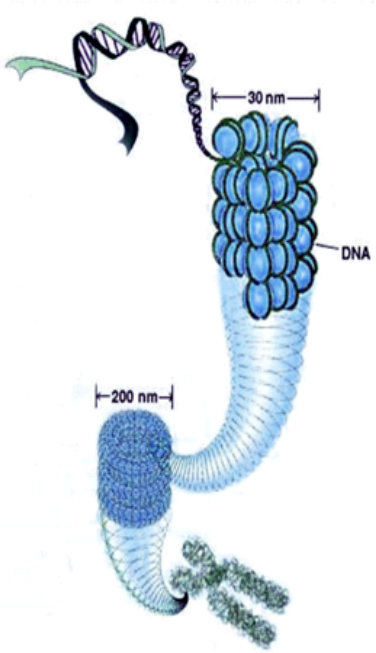
Listeria **infection induces the degradation of UBC9 via LLO**

Listeria infection and histone modifications

Listeria monocytogenes : a cell biologist

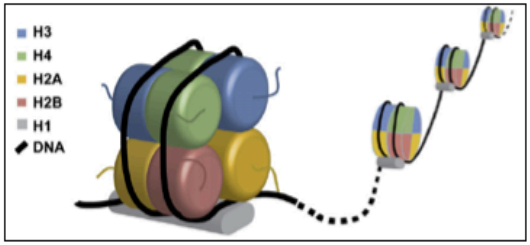


Chromatin and nucleosome structure

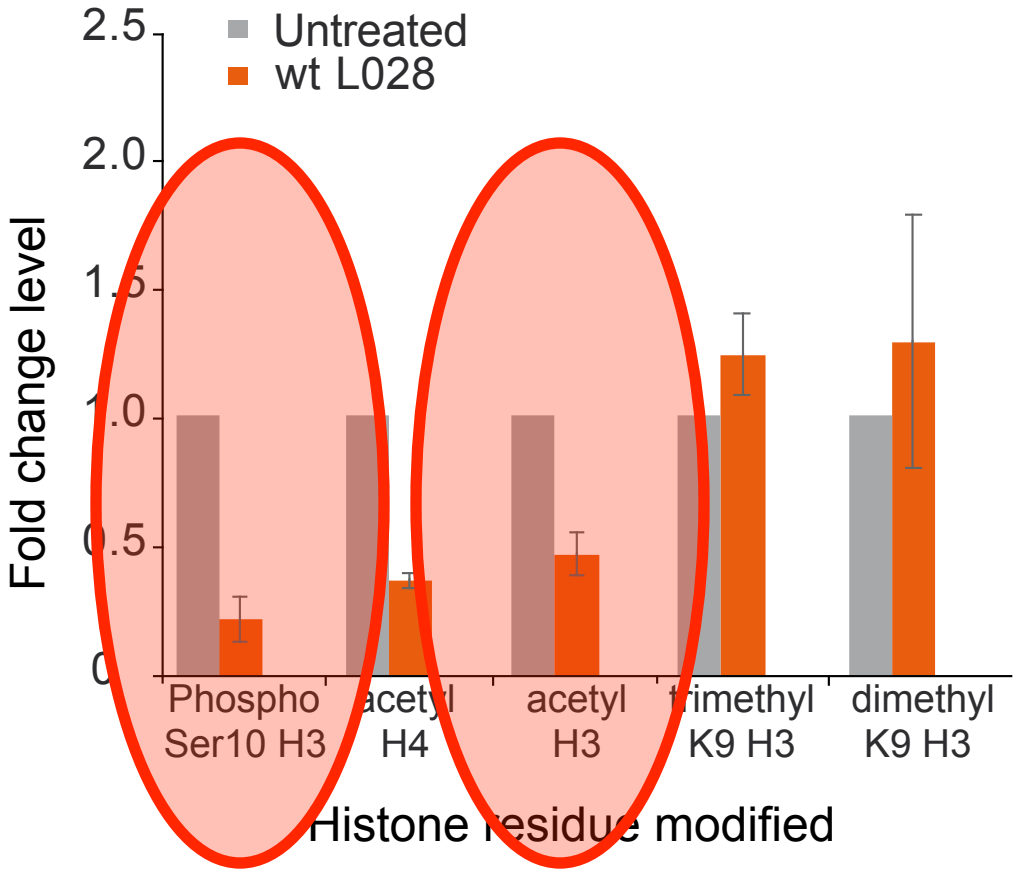


Chromatin:

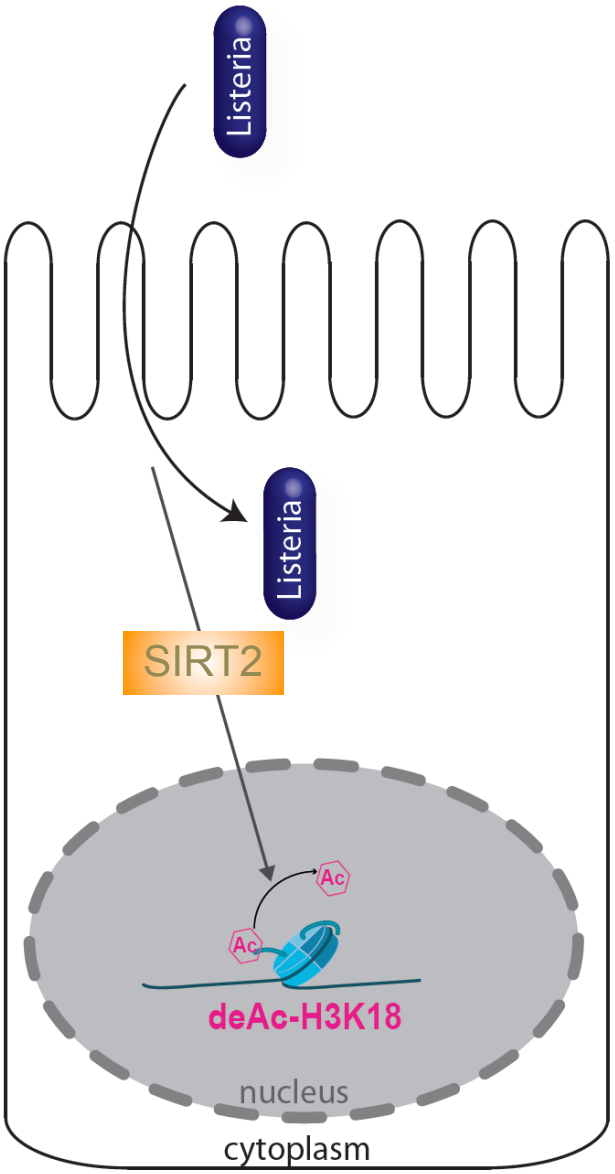
- Highly architectured structure important for packaging DNA into a compact structure
- The basic unit is the nucleosome



L. monocytogenes induces specific histone modifications



Model for H3 deacetylation at K18

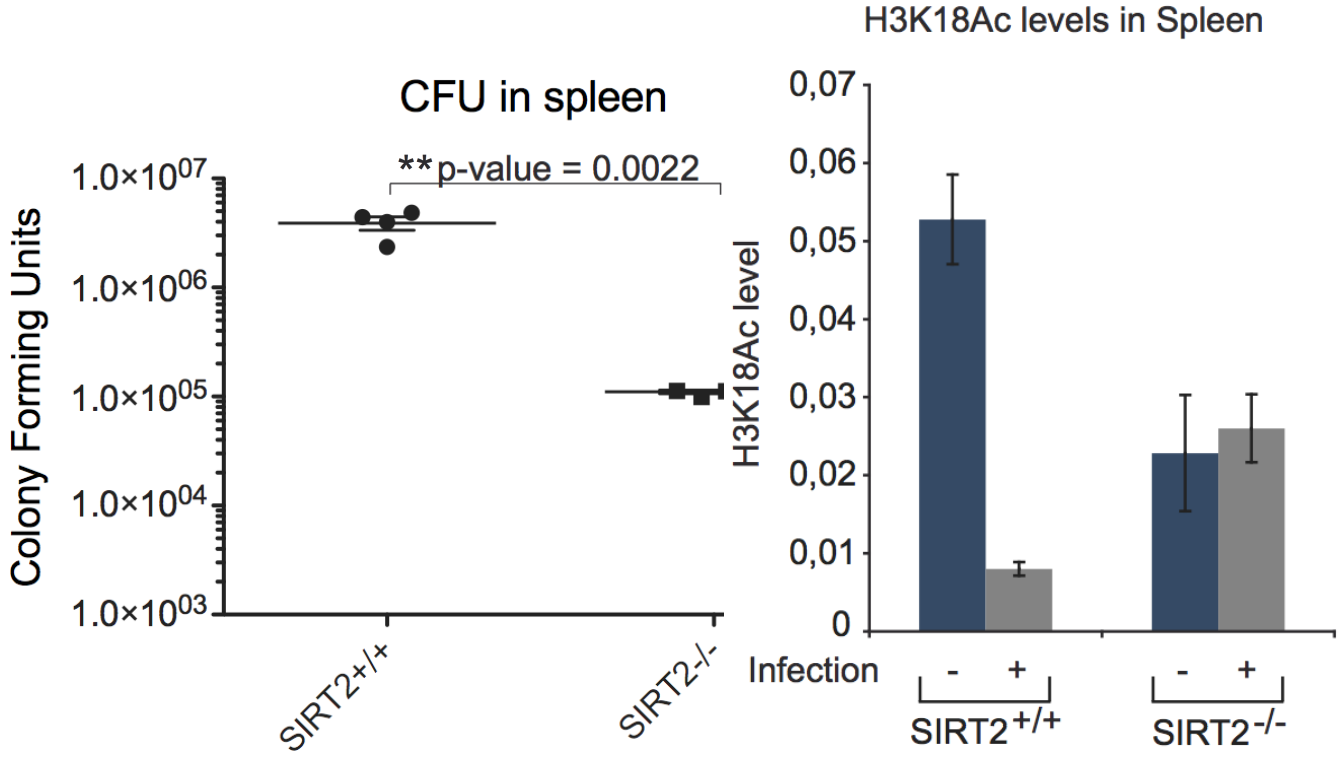


- **SIRT2**, which is normally present in the cytoplasm of interphasic cells, relocalizes to the chromatin upon infection

- Infection "activates" SIRT2 to induce H3 deacetylation and gene repression.

Infection is lower in SIRT2$^{-/-}$ mice

72h EGD IV-infection of C57BL6 male mice

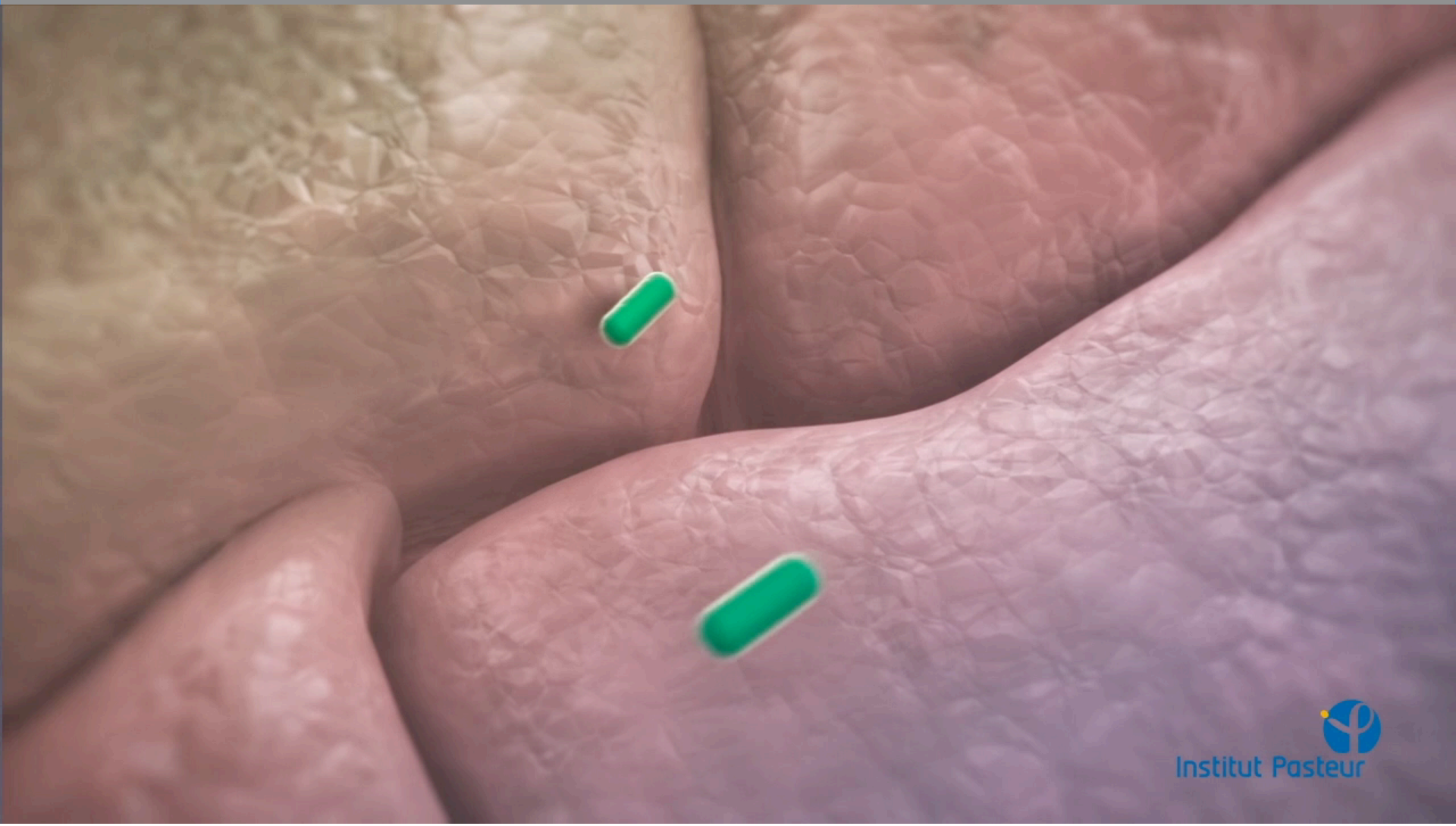


The histone modifier Sirt2 is essential for infection

Take home messages

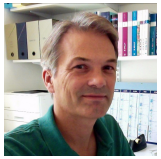
- *Listeria* virulence genes are thermoregulated via a **RNA thermosensor**, a special type of RNA riboswitch responding to temperature
- The internalin E-cadherin interaction mediates crossing of the intestinal barrier, as demonstrated by infection of **transgenic human Ecad mice**
- The study of *Listeria* entry into cells has led to a change in paradigm concerning **clathrin**
- ActA has been instrumental to discover and characterize **the role of Arp2/3** in actin-based motility
- *Listeria* uses tricks of commensals such as a **bile salt hydrolase**
- Mitochondrial dynamics is critical for infection.Infection affects **mitochondria**
- Infection affects **epigenetic/chromatin regulators**

Listeria cell infection process



Acknowledgements

IP, Paris



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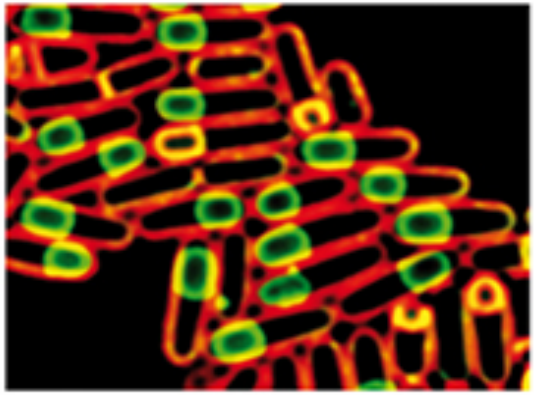
Des microbiotes aux CRISPR



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