Epigenetic reprogramming by *Listeria monocytogenes*

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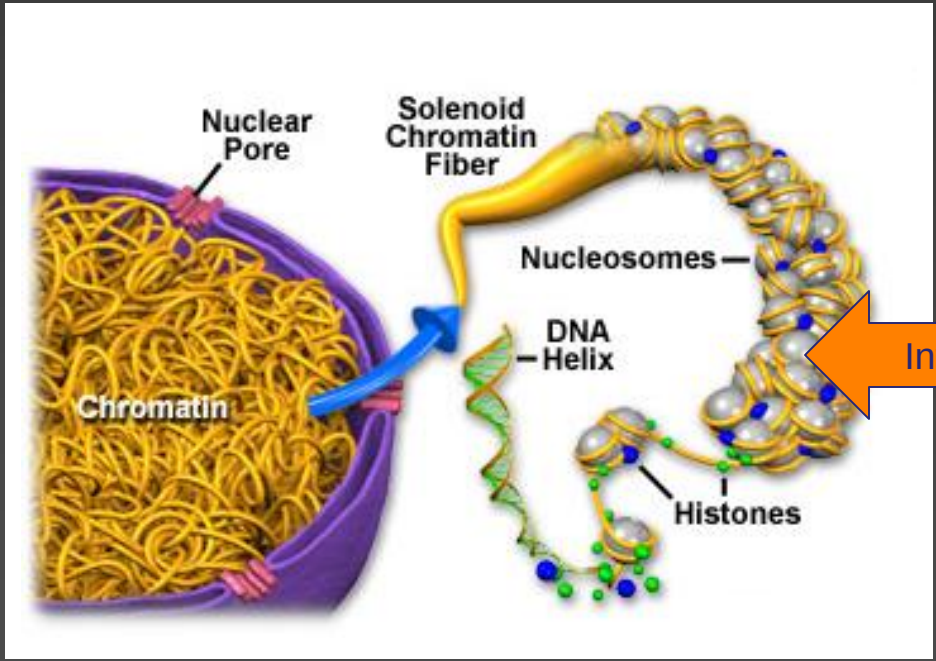
Fondation Merieux – June 2015

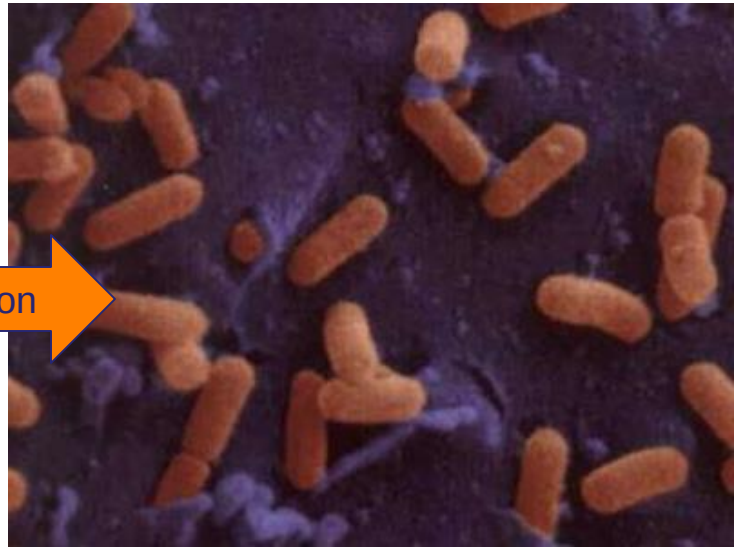
FOR RESEARCH, FOR HEALTH,
FOR OUR FUTURE

Institut Pasteur

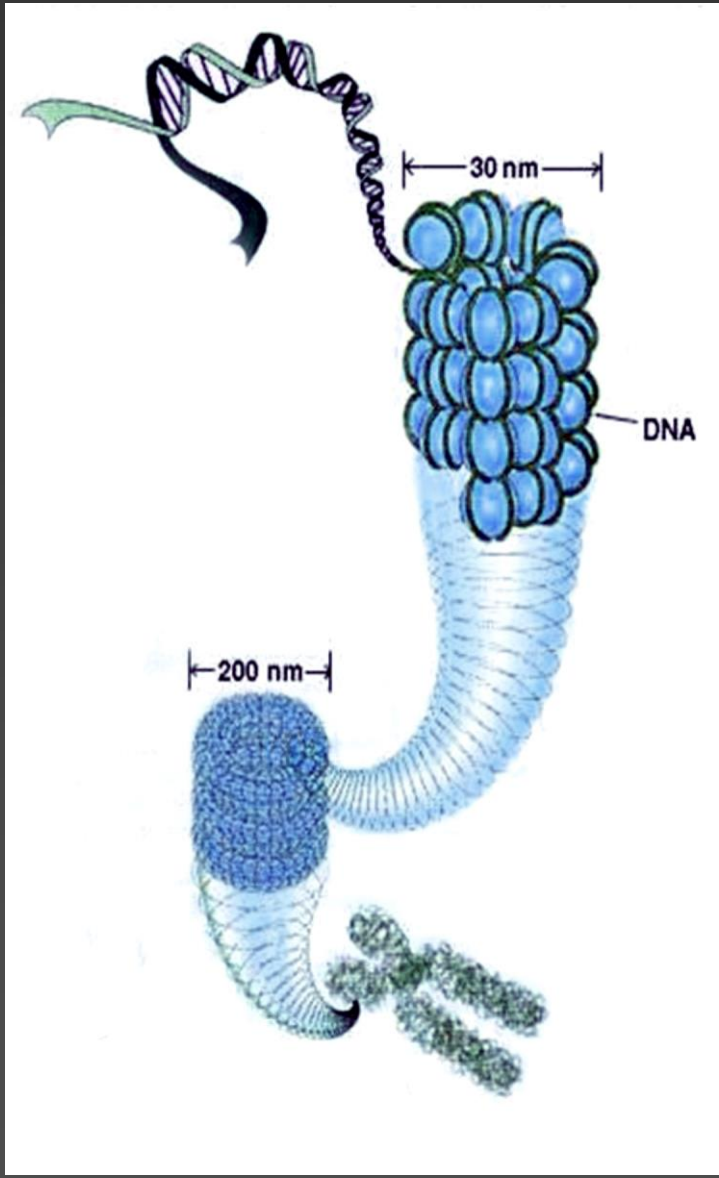
Basic biological mechanism of what occurs during infection



Interaction

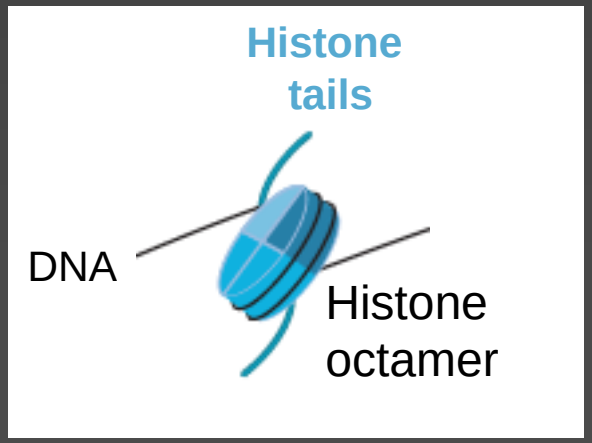


Chromatin and nucleosome structure

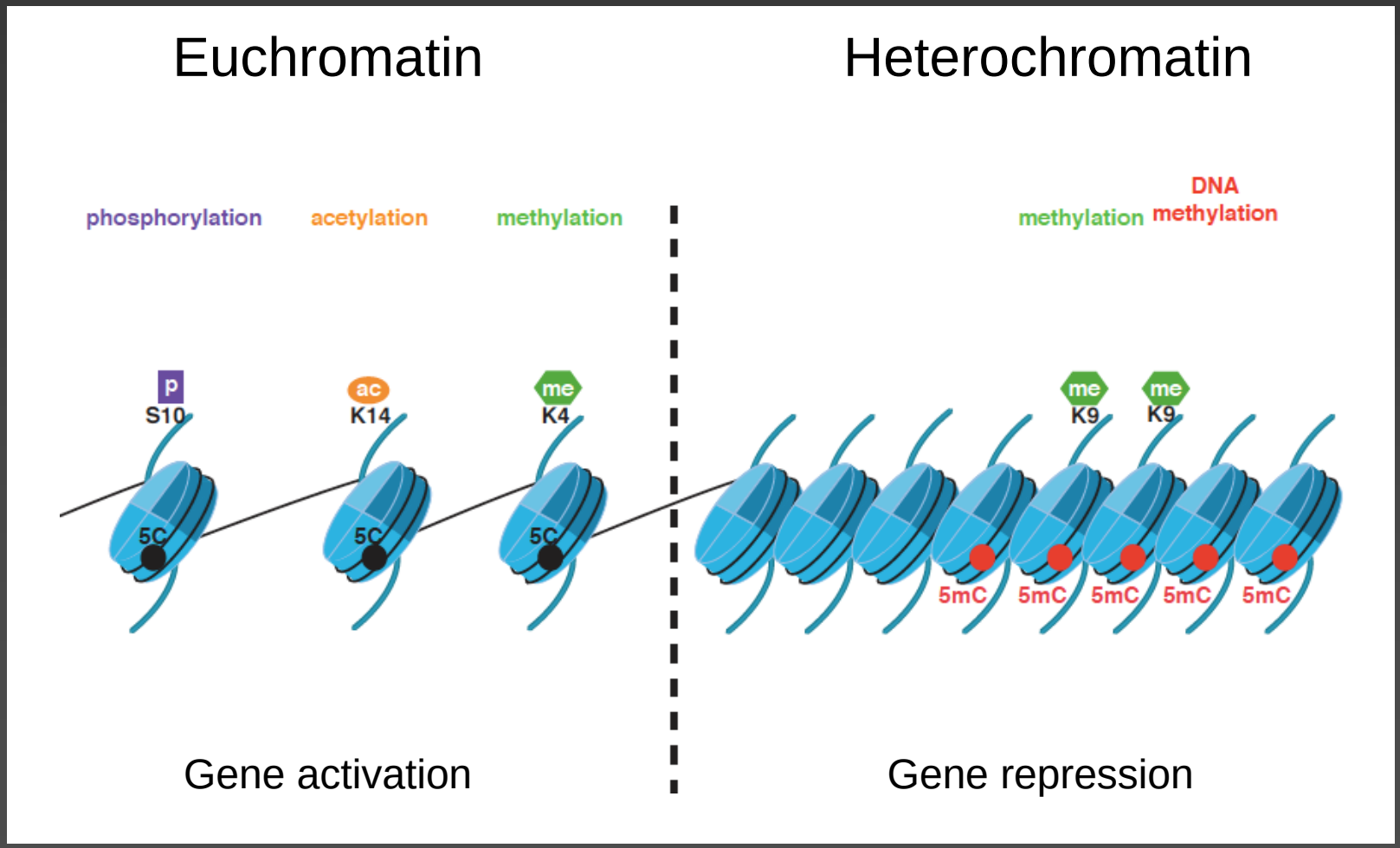


Chromatin:

- Highly architectured structure important for packaging DNA in the nucleus
- The basic unit is the nucleosome

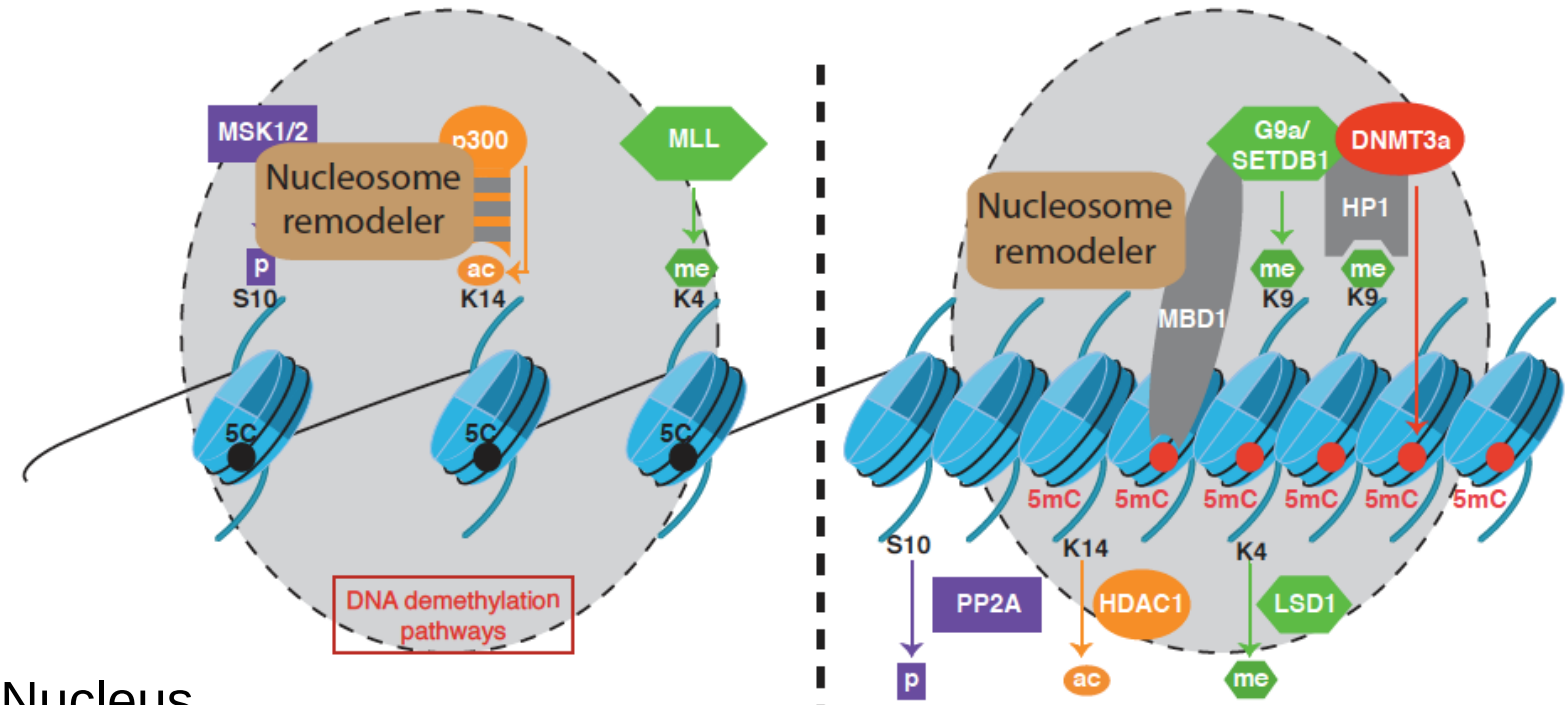


Chromatin structure depends on histone modifications and DNA methylation



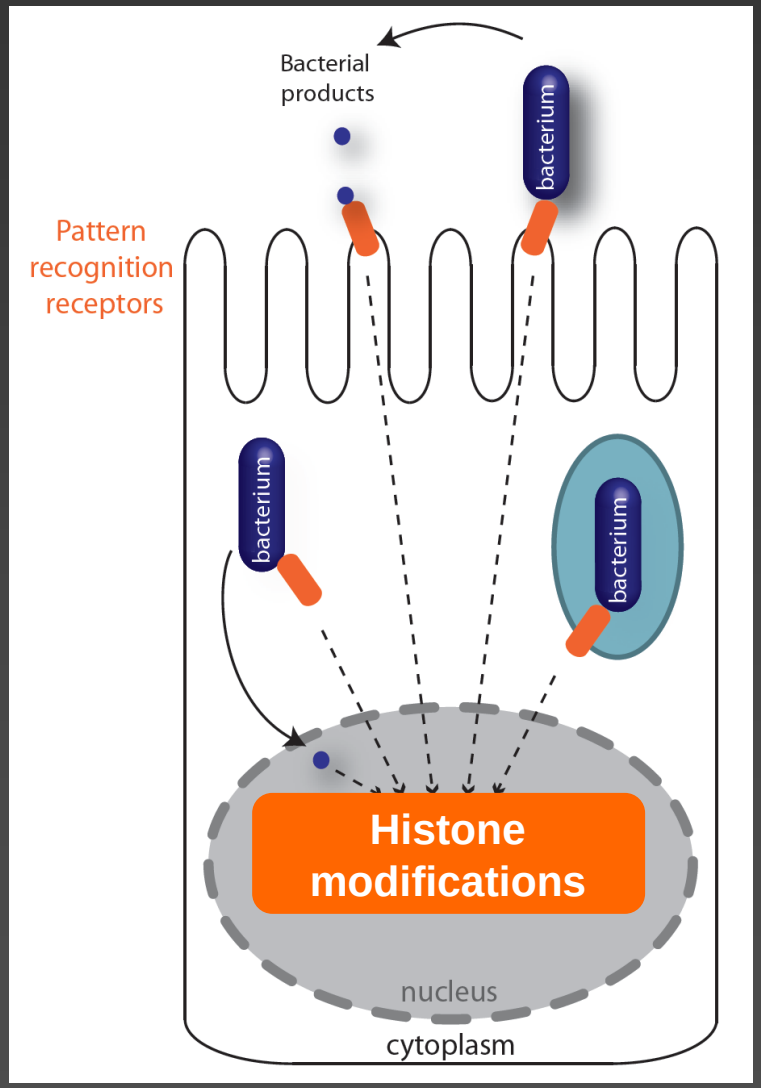
The chromatin (« epigenetic ») language

Environmental stimuli → Signal transduction pathways

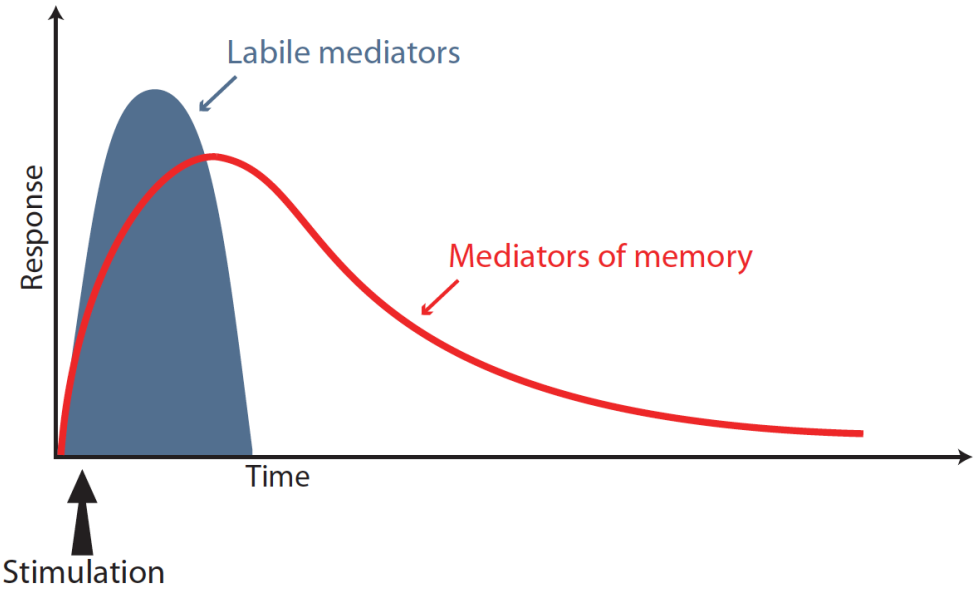


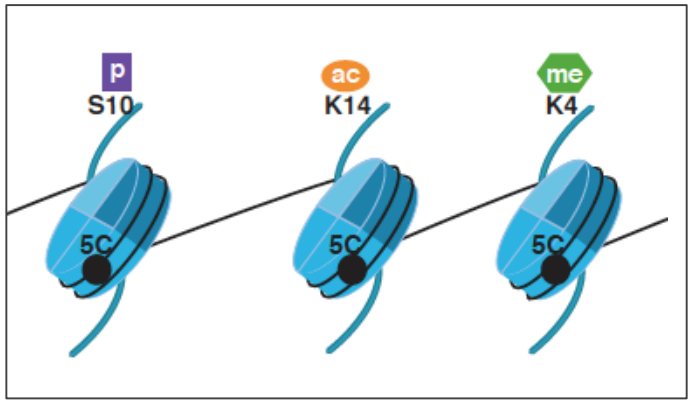
Nucleus

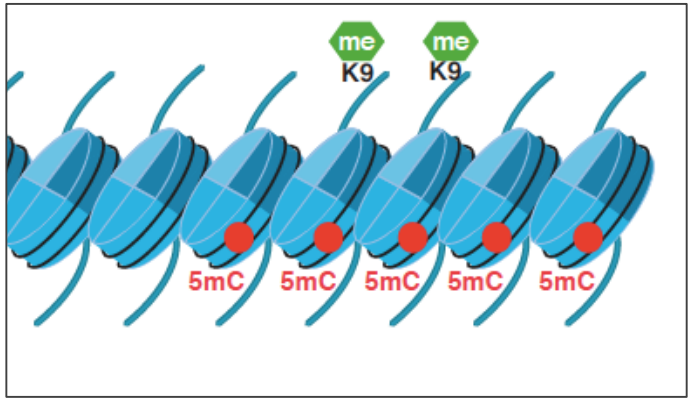
Pathogens alter the transcriptional program of their host



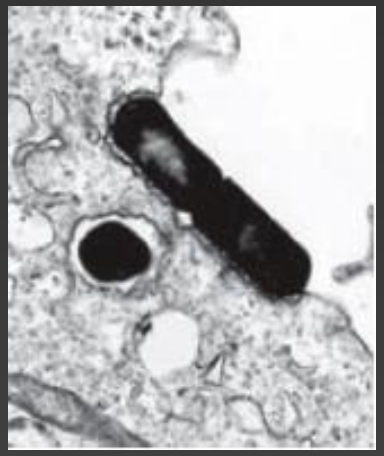
The lasting potential of histone modifications

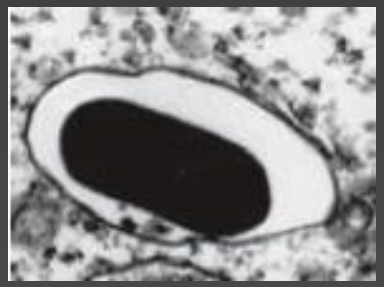


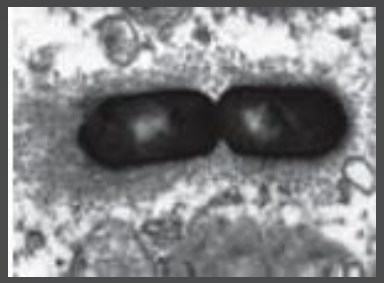


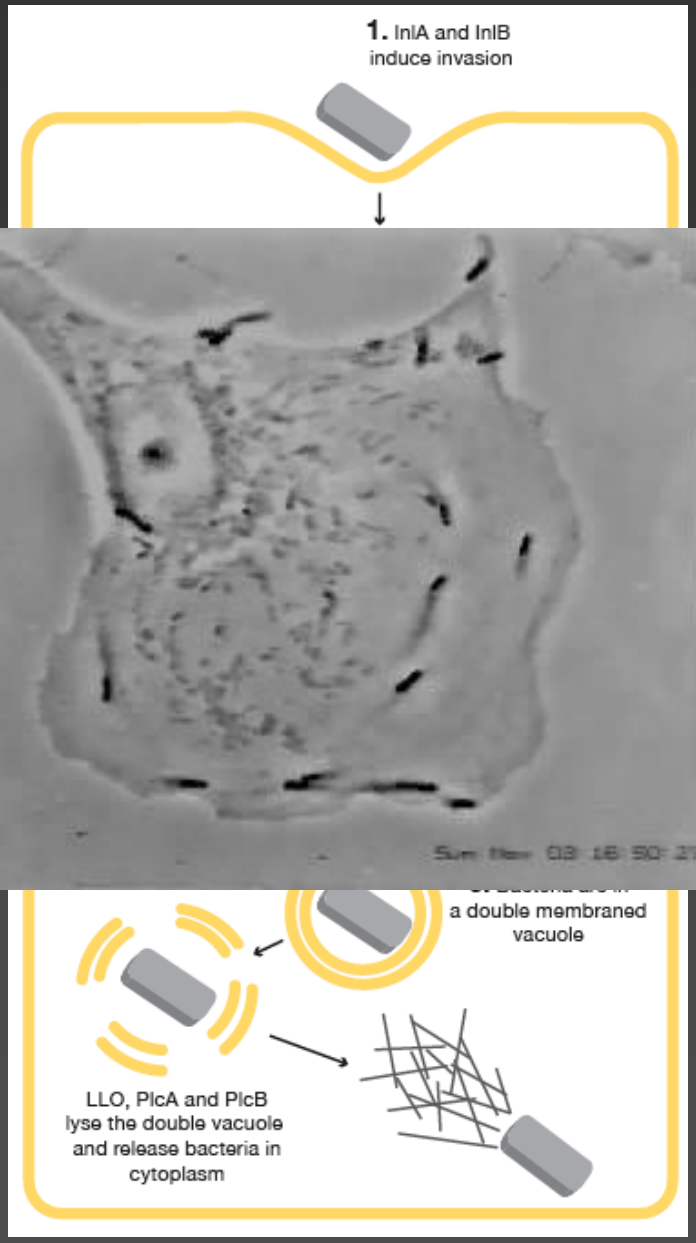


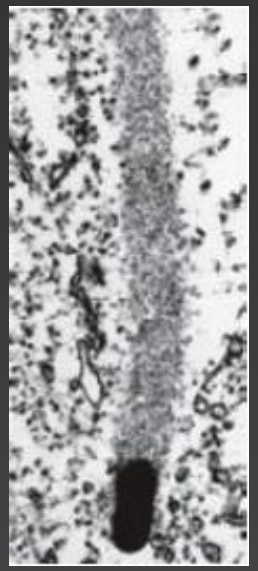
Listeria : a model of invasive and intracytosolic pathogen

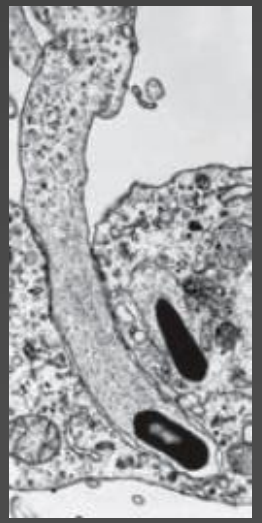




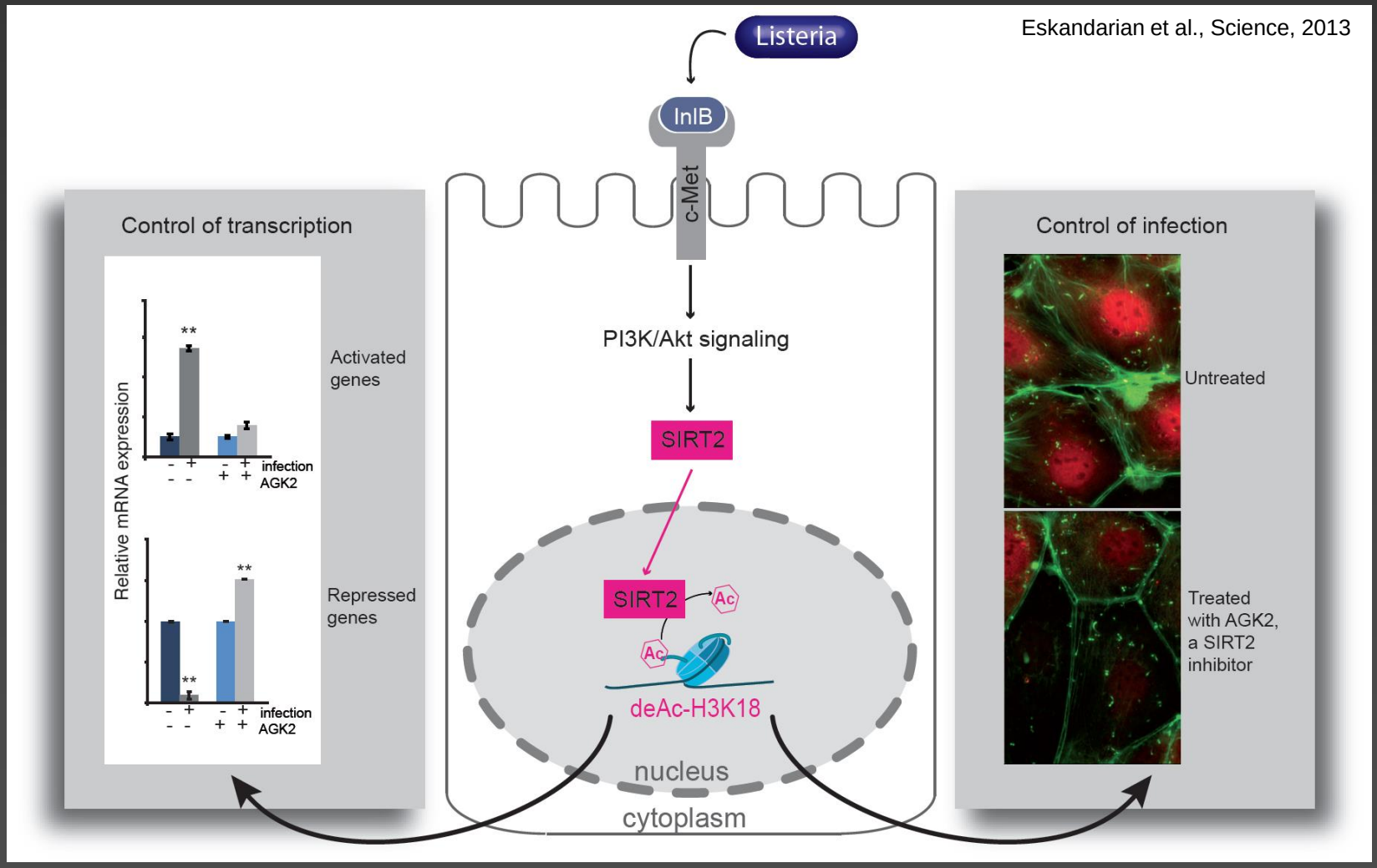




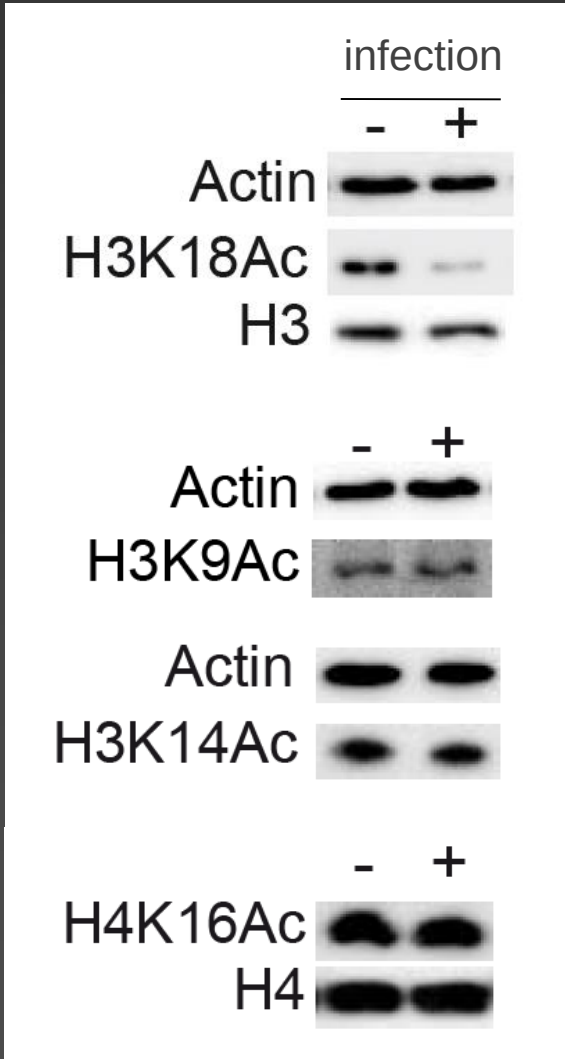


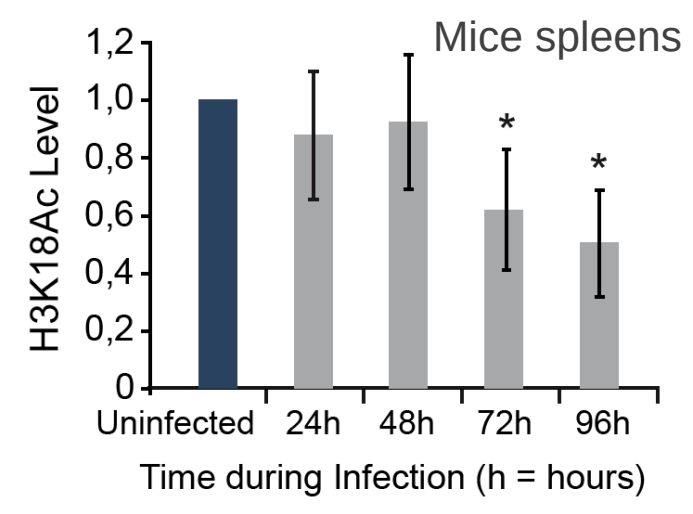


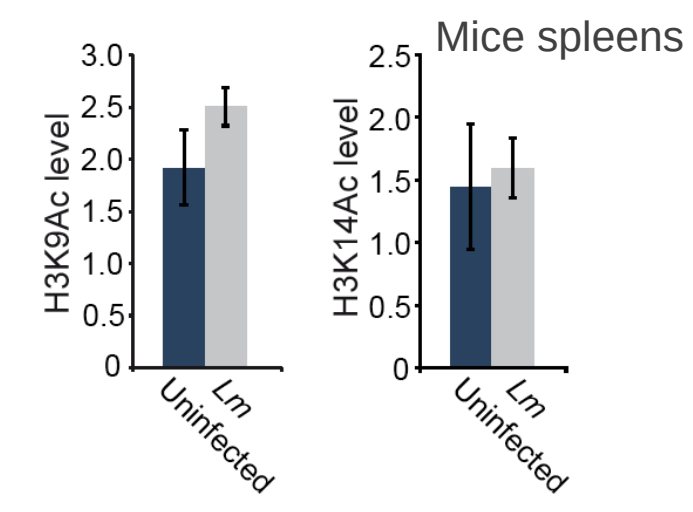
Host deacetylase SIRT2 is coopted by *Listeria* to induce deacetylation of histone H3K18



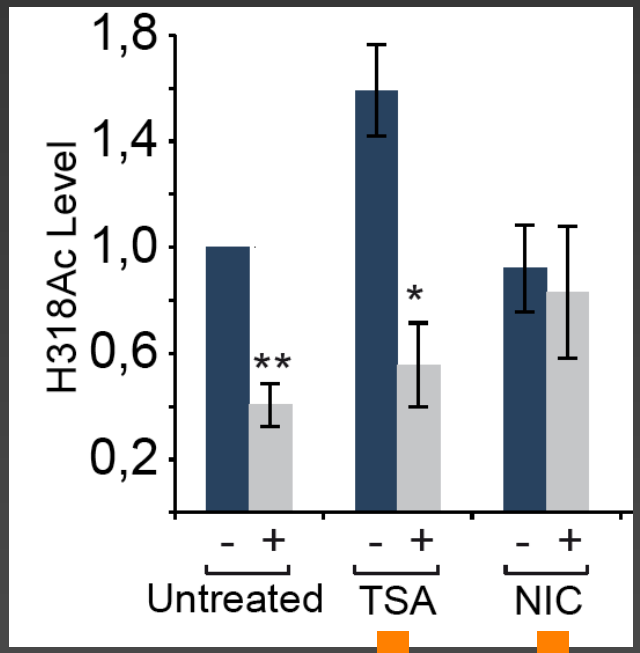
Listeria monocytogenes induces H3K18 deacetylation during infection *in vitro*, and *in vivo*

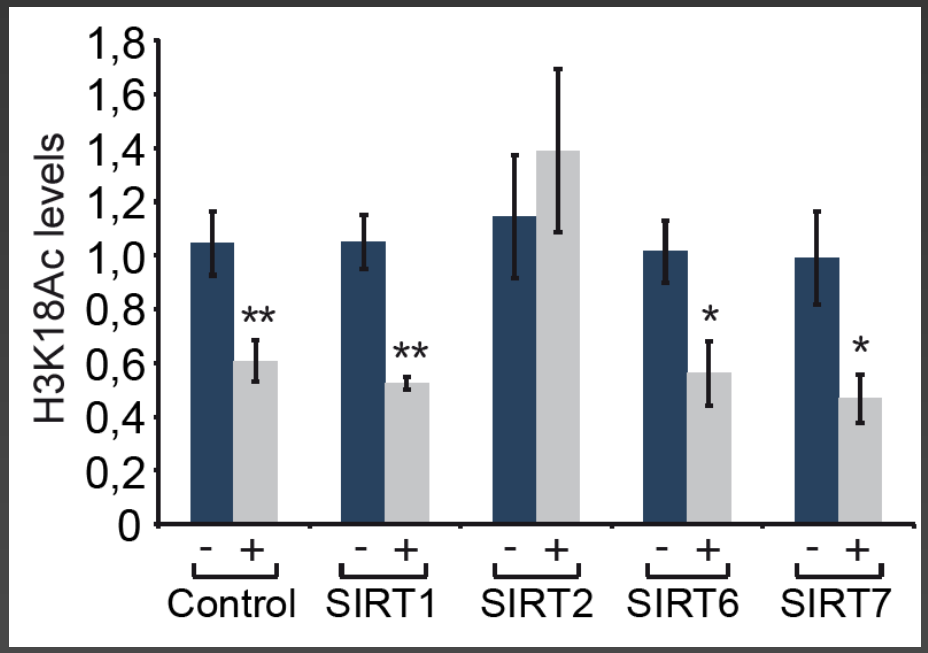




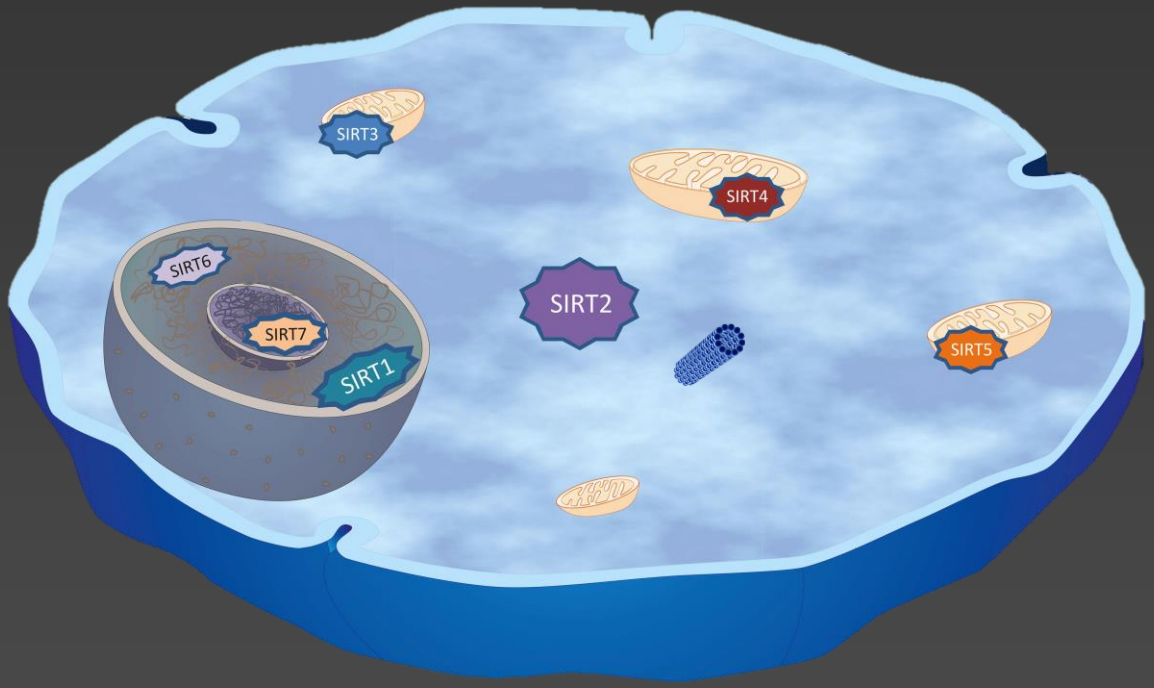


Infection-induced H3K18 deacetylation is dependent on SIRT2





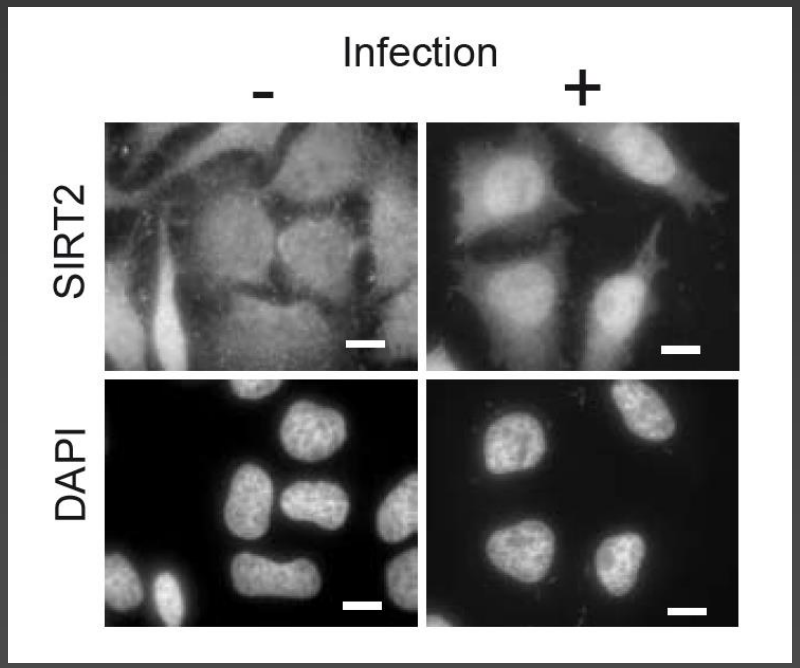
Sirtuins, are protein deacetylases dependent on nicotine adenine dinucleotide (NAD)

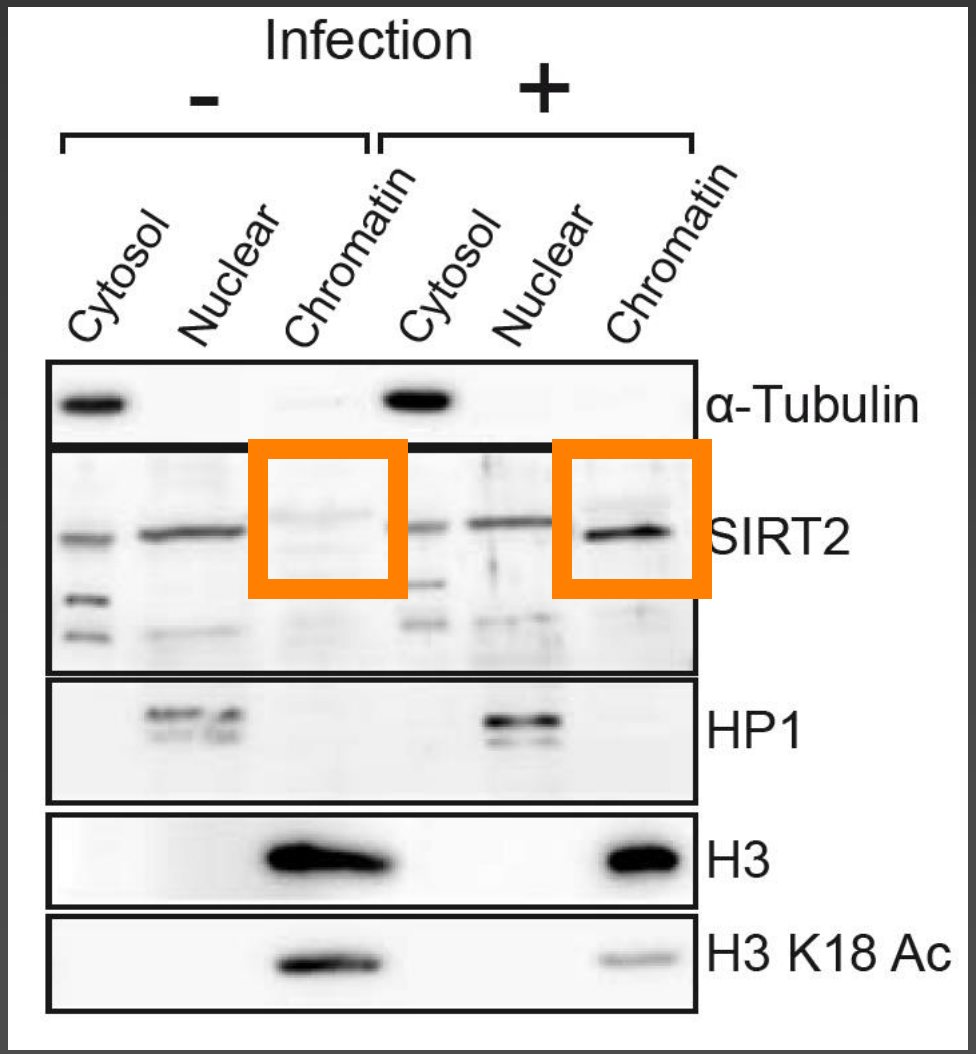


Modified from: Oliveira *et al*. Front Pharmacol. 2012

- SIRT2 is cytoplasmic and associates with the microtubule network
- SIRT2 shuttles between cytoplasm and nucleus (mechanism and role unknown)
- The best characterized target of SIRT2 is α-tubulin
- SIRT2 has an important role in regulation of aging and cancer

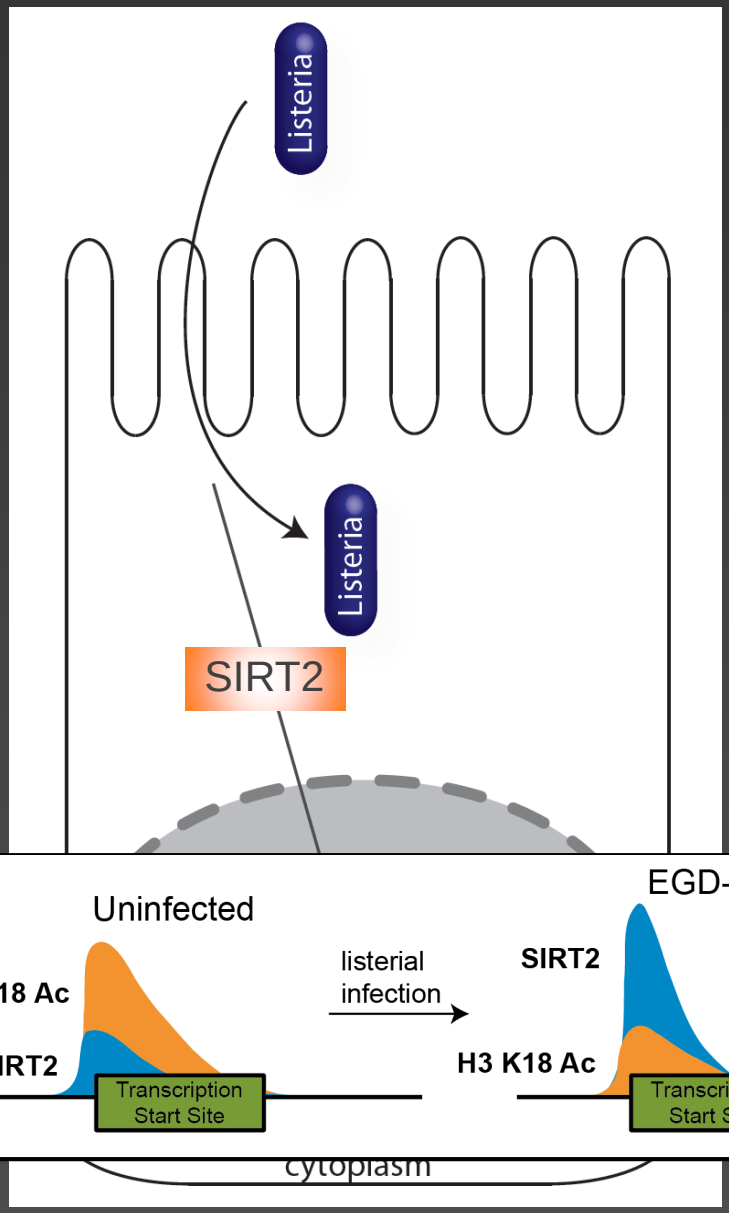
SIRT2 is targeted to the chromatin during infection





Where H3K18 deacetylation occurs…

Model

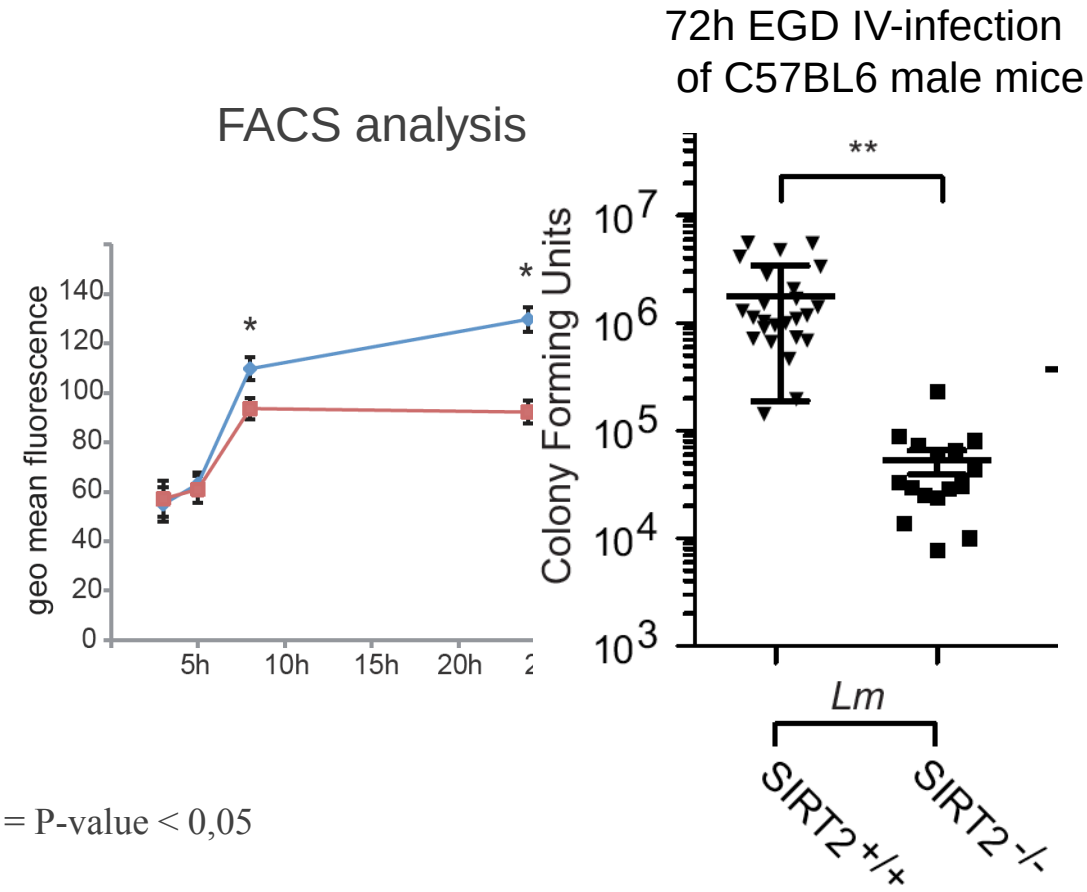


- SIRT2, which has only been characterized in the cytoplasm of interphase cells, relocalizes to the chromatin upon infection
- Gene repression during infection is almost entirely dependent on SIRT2
- Infection targets SIRT2 to transcription start sites of repressed genes where H3K18 is deacetylated

Defines a new role for SIRT2 in transcriptional regulation

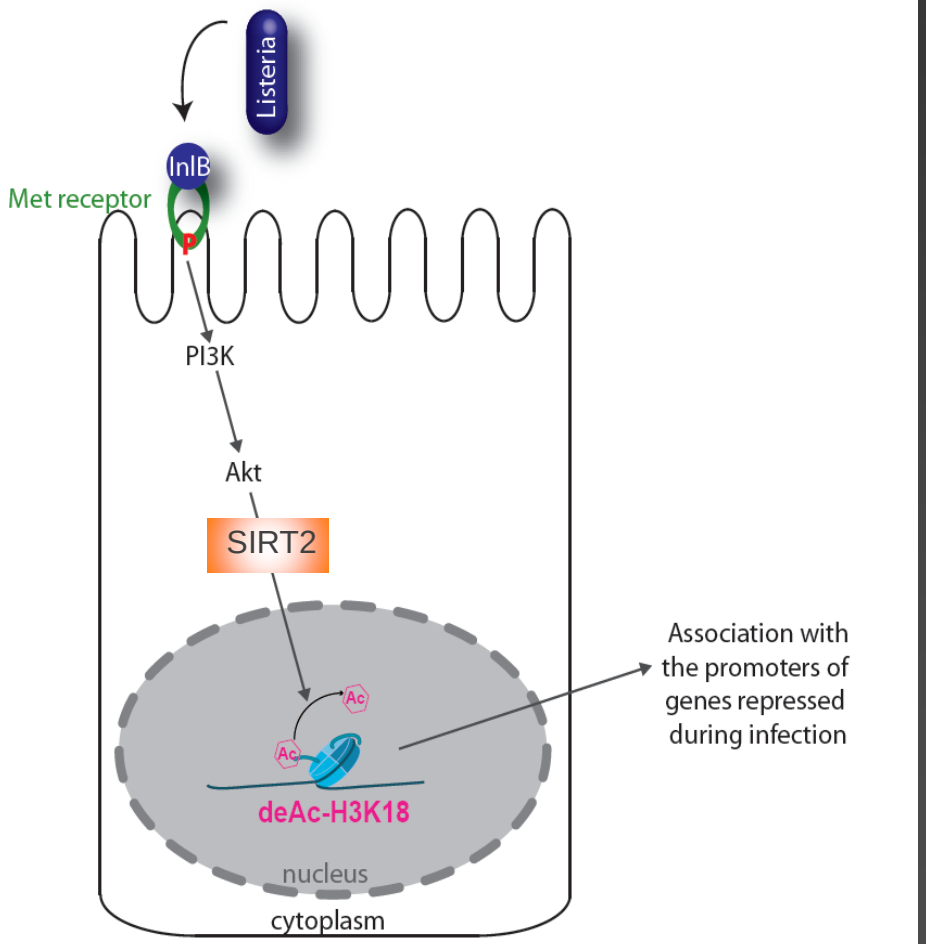
Eskandarian et al., Science, 2013

SIRT2 activity is necessary for infection with *Listeria monocytogenes*



* = P-value < 0,05

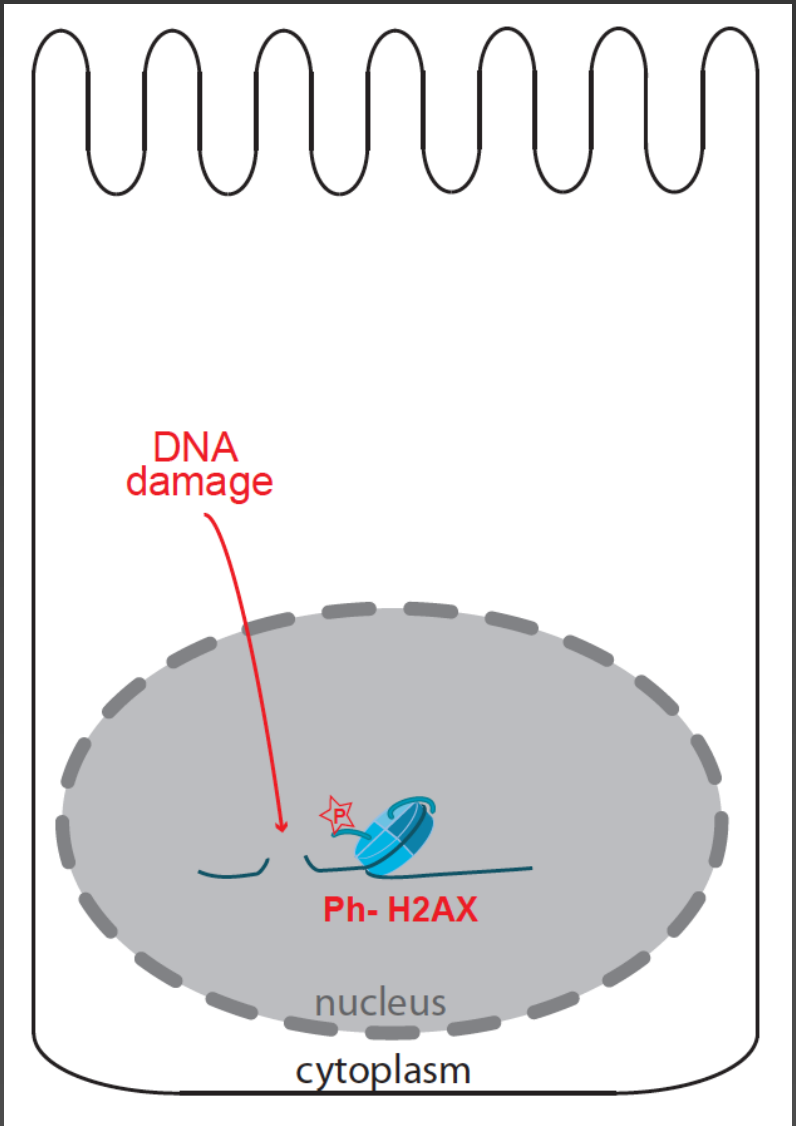
Host deacetylase SIRT2 is coopted by *Listeria* to induce deacetylation of histone H3K18



Eskandarian et al., Science, 2013

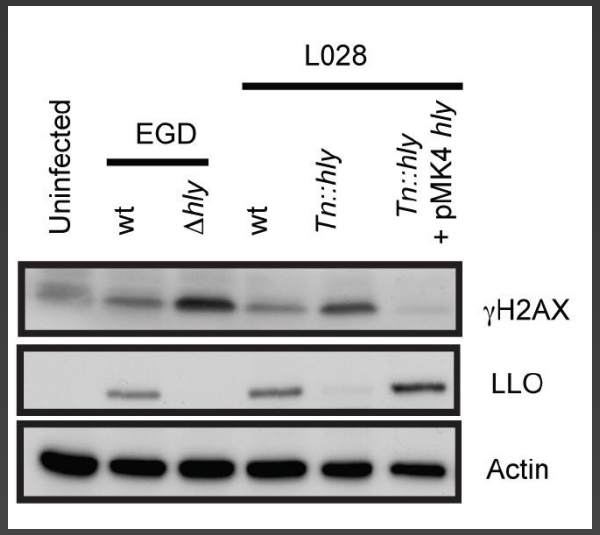
- *L. monocytogenes* induces specific deacetylation of H3K18 in vitro and in vivo
- Deacetylation is mediated by the host deacetylase SIRT2
- SIRT2 is relocalized during infection from cytoplasm to nucleus upon activation of the Met receptor
- SIRT2 is targeted to transcription start sites of genes repressed during infection where H3K18 is deacetylated
- SIRT2 activity is necessary for a productive infection both in vitro and in vivo

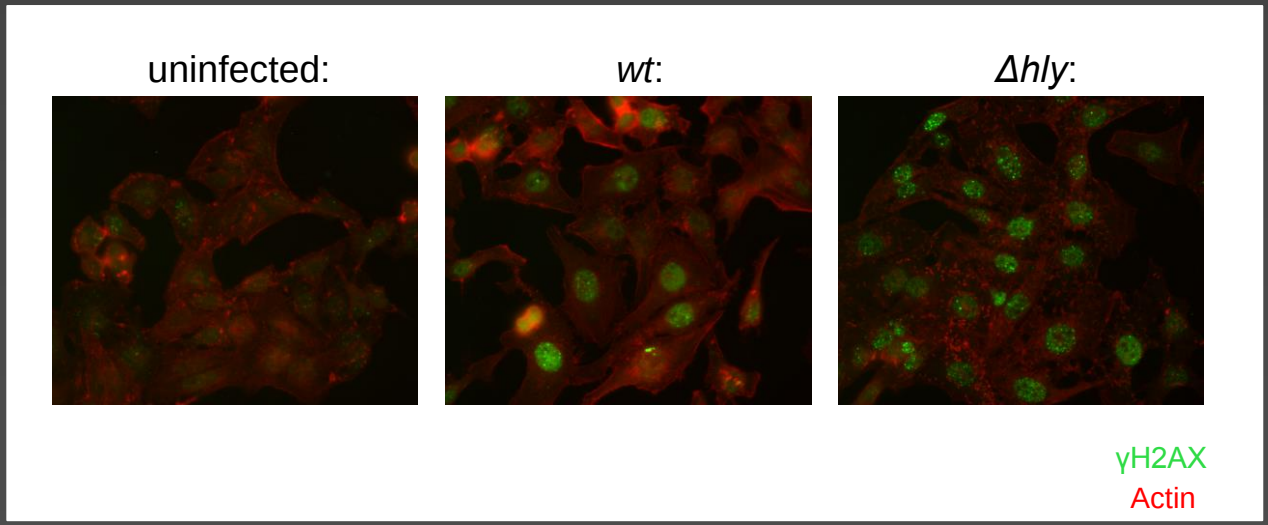
Listeria monocytogenes dampens the DNA damage response



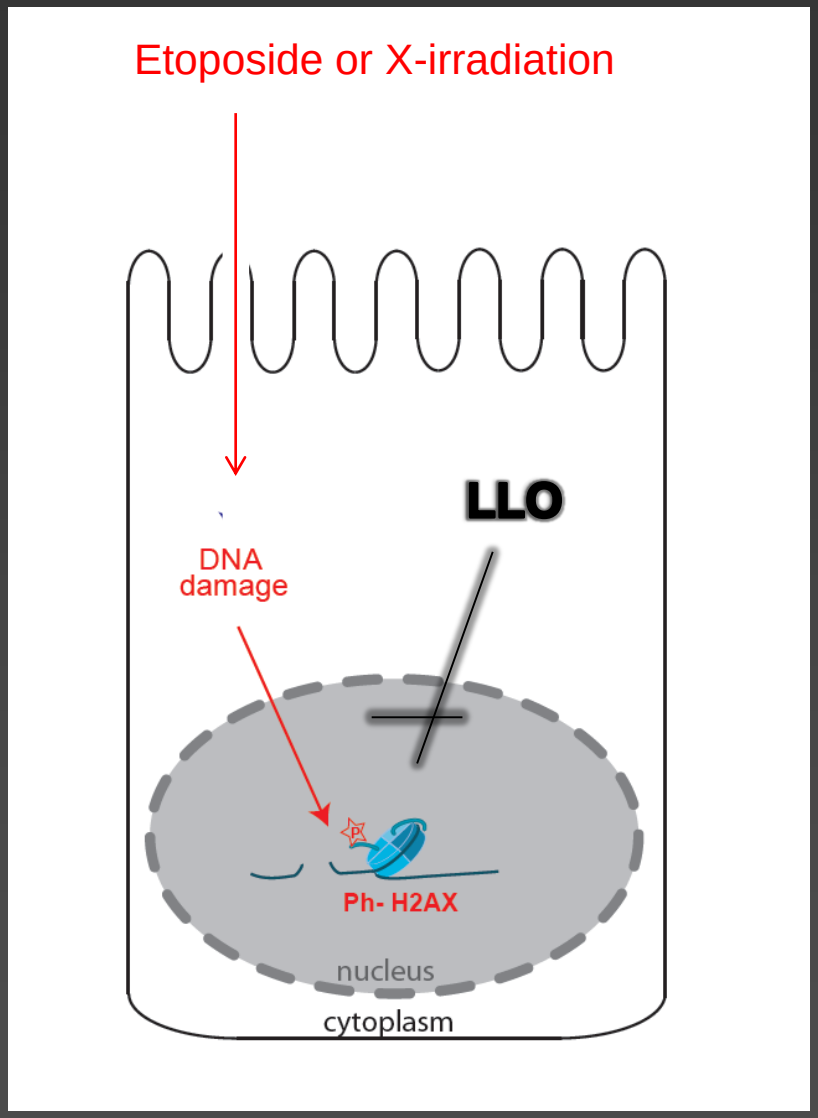
- Ionizing radiation, stress etc. induces DNA breaks
- Histone H2A variant X becomes phosphorylated at serine 139 (γH2AX) upon DNA damage
- γH2AX is the most sensitive marker used to examine DNA damage

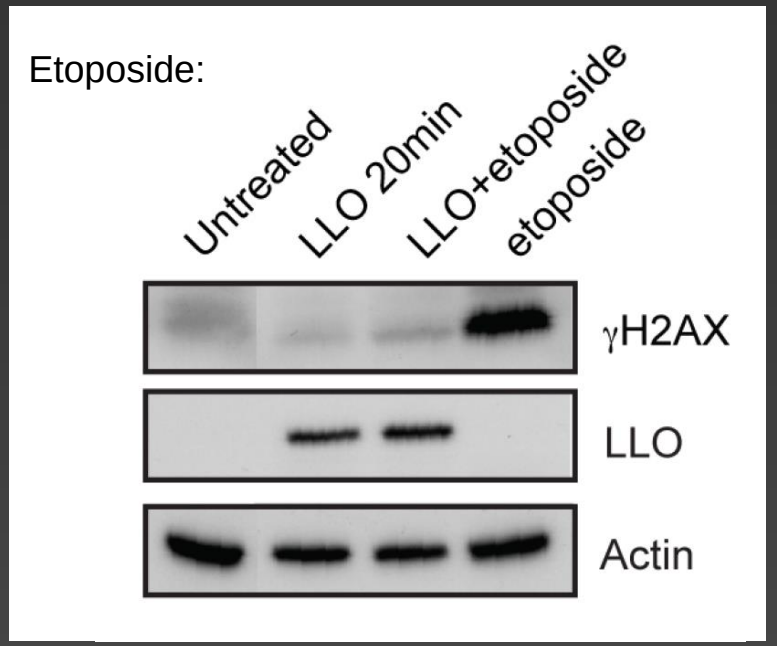
Listeria monocytogenes Δhly induces more γH2AX than wild type

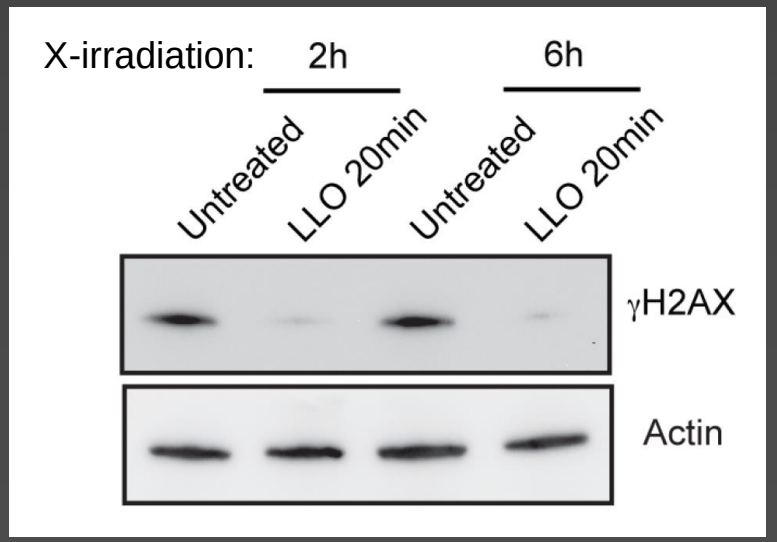




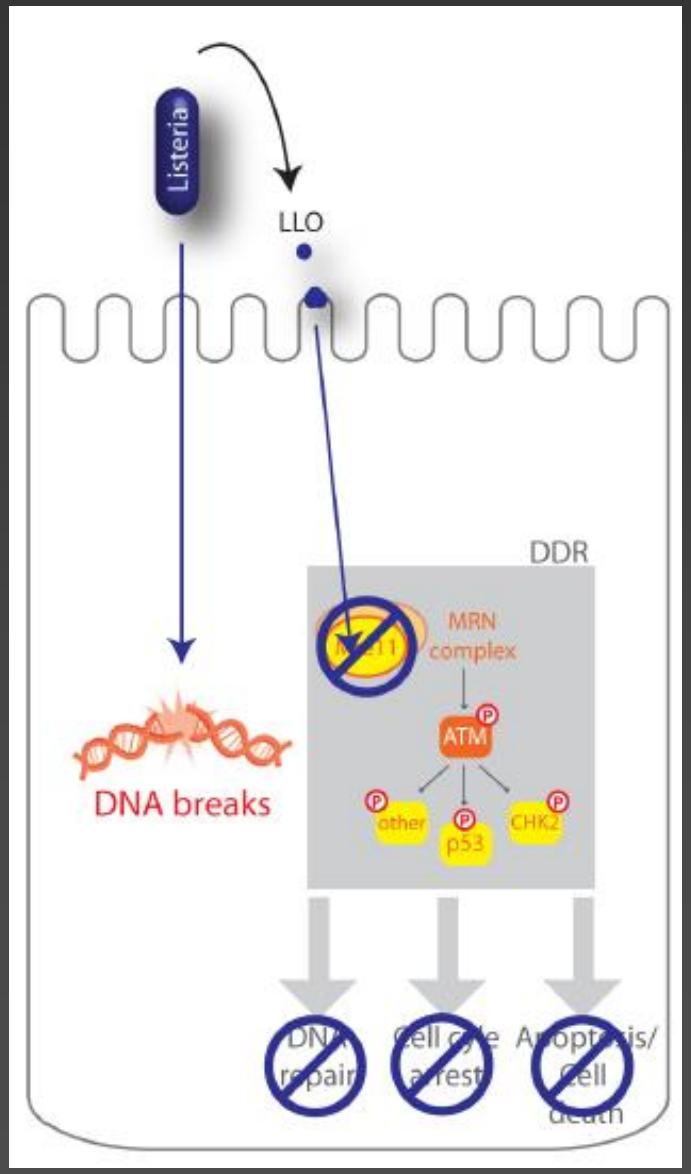
LLO impairs activation of the DNA damage response







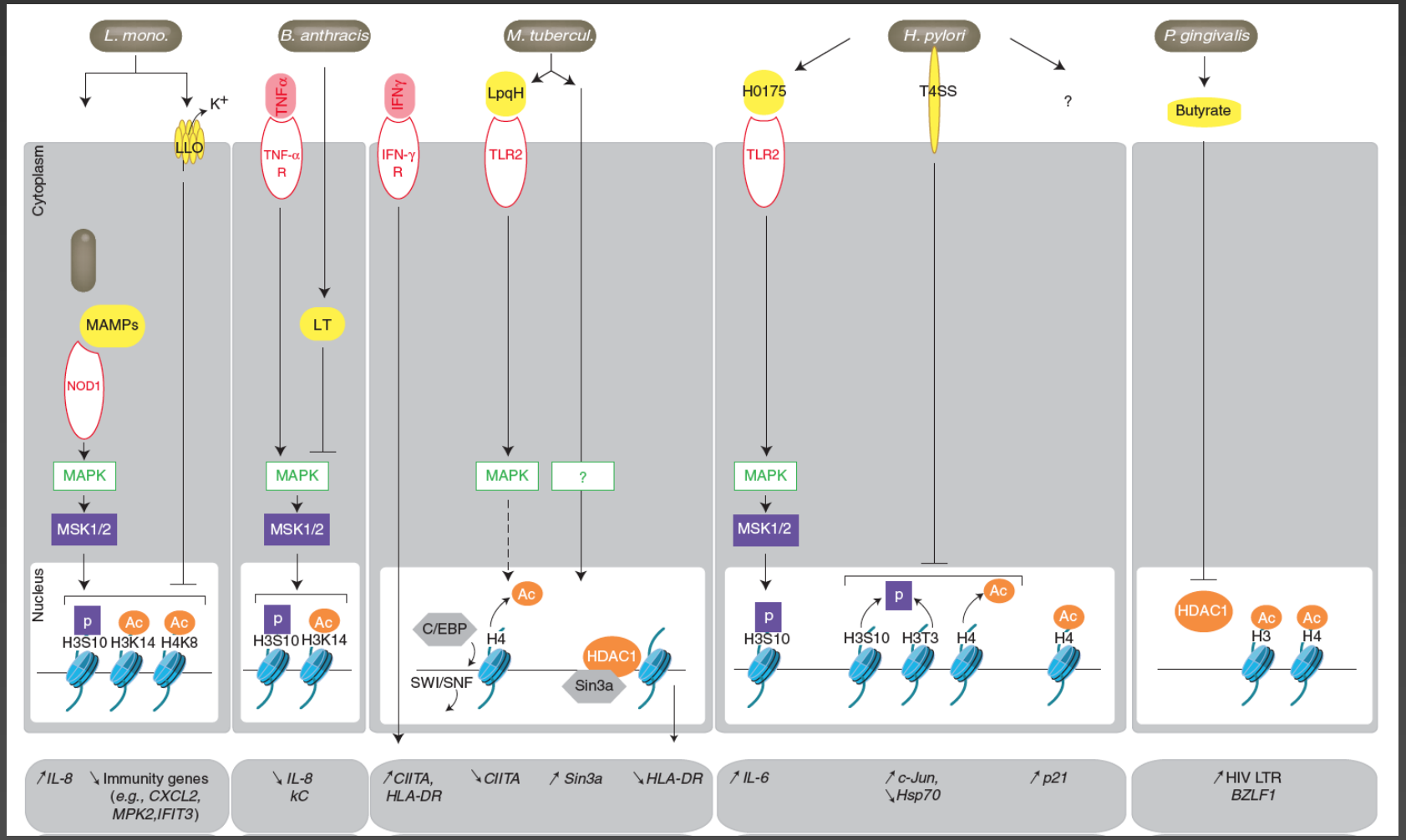
Listeria monocytogenes dampens the DNA damage response



- *L. monocytogenes* induces DNA breaks during infection
- The DNA damage response is dampened by LLO during infection
- LLO degrades the major sensor of DNA breaks, Mre11
- Dampening of the DDR is important for a productive infection

Samba-Louaka al., Plos Path, 2014

Conclusions and perspectives



Epigenetic memory of infection

Bierne, Hamon and Cossart, CSHPM, 2012

Aknowledgements

Pascale Cossart

SIRT2

Alexander Eskandarian

Francis Impens

Marie-Anne Nahori

Collaborators:

Guillaume Soubigou (Institut Pasteur)

Jean-Yves Coppée (Institut Pasteur)

Constructs:

Brian North (Harvard Medical School)

DNA damage

Ascel Samba-Louaka

Jorge Pereira

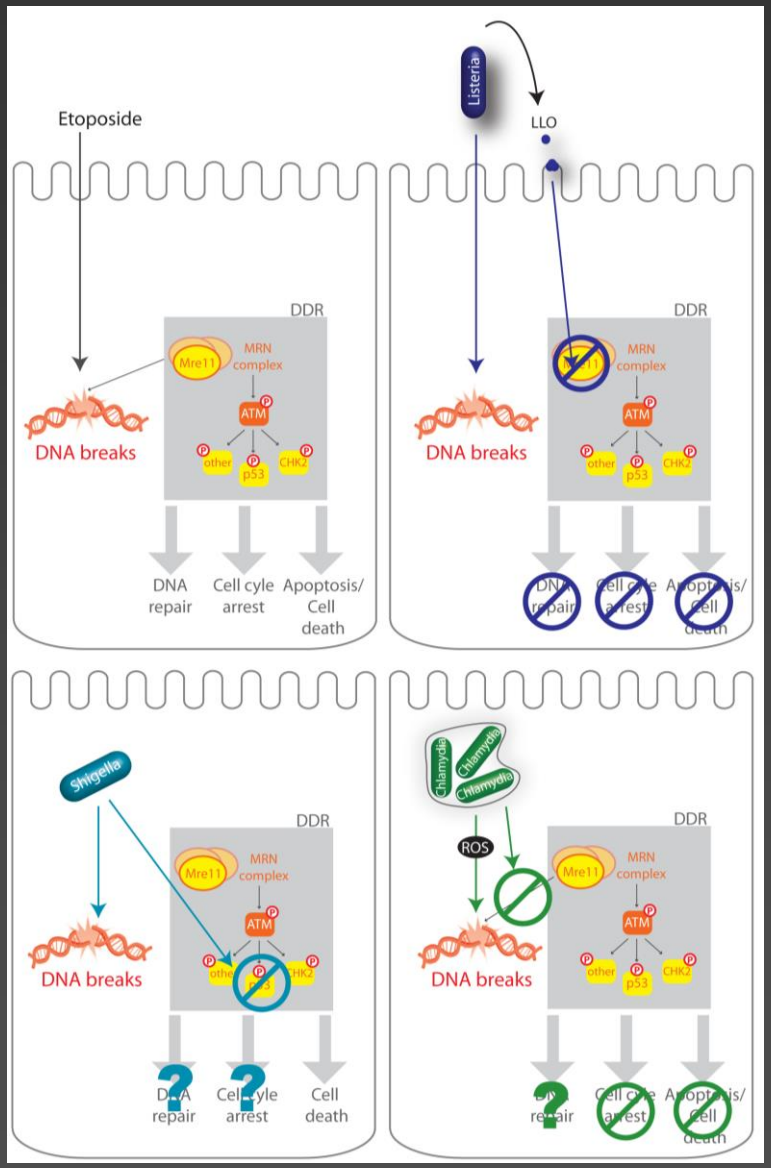
Collaborators:

Ludovic Deriano (Institut Pasteur)

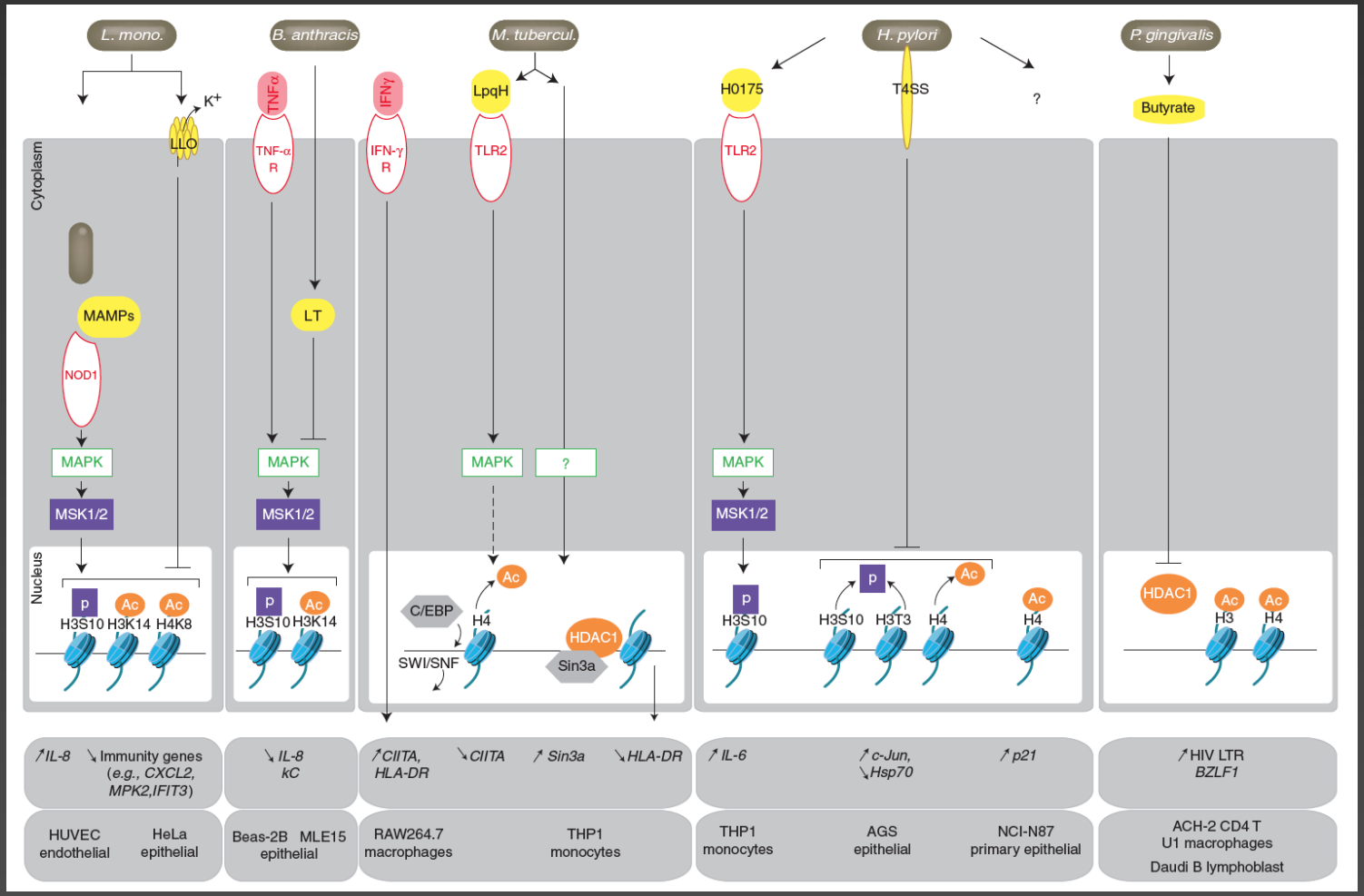
Cell Lines:

John Petrini (Memorial Sloan-Kettering cancer center)

The DNA damage response during infection



Bacterial signaling to histones and downstream effects



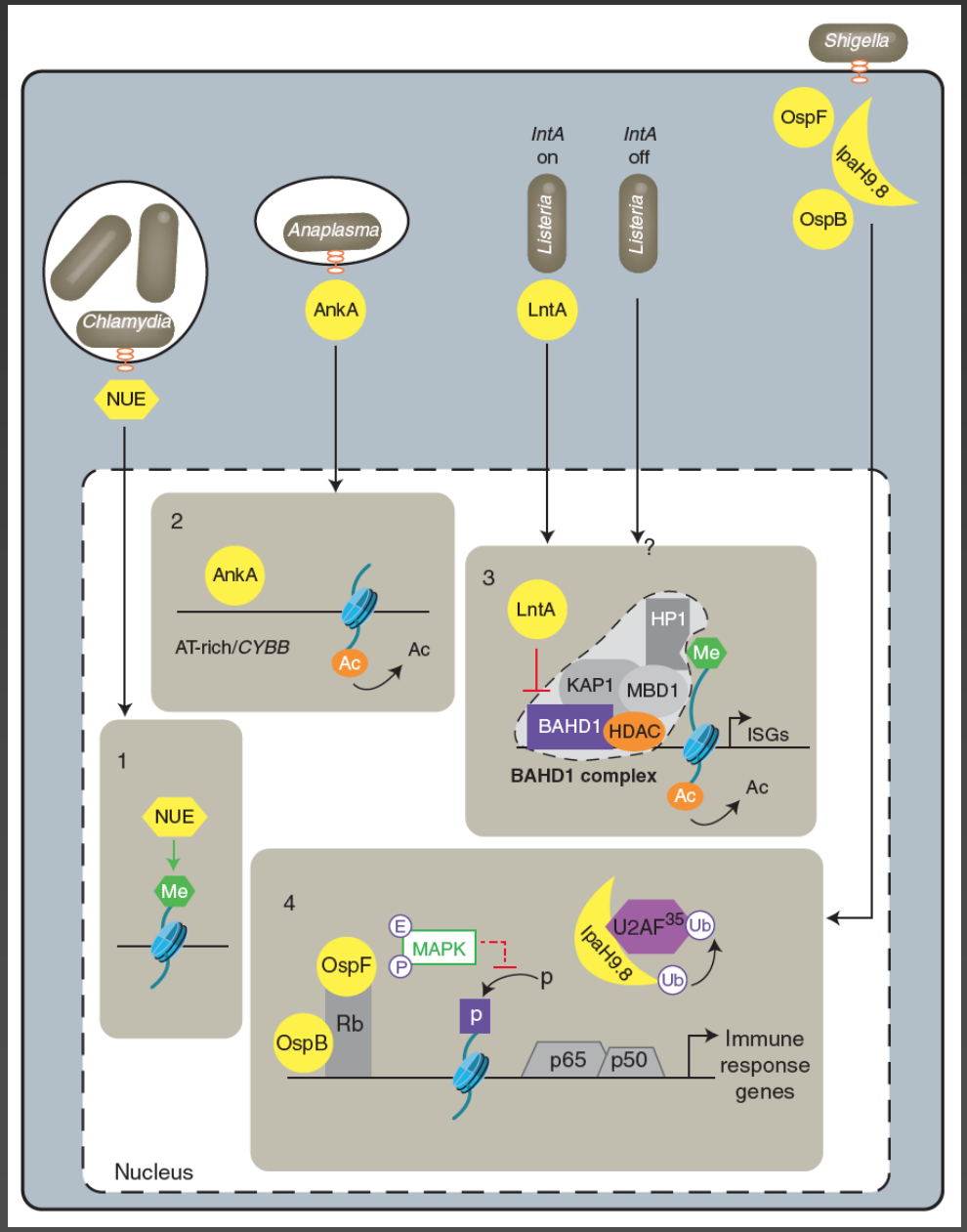
Reviews on the topic:

H. Bierne, M. Hamon, P. Cossart, *Cold Spring Harb Perspect Med* **2**, (2012).

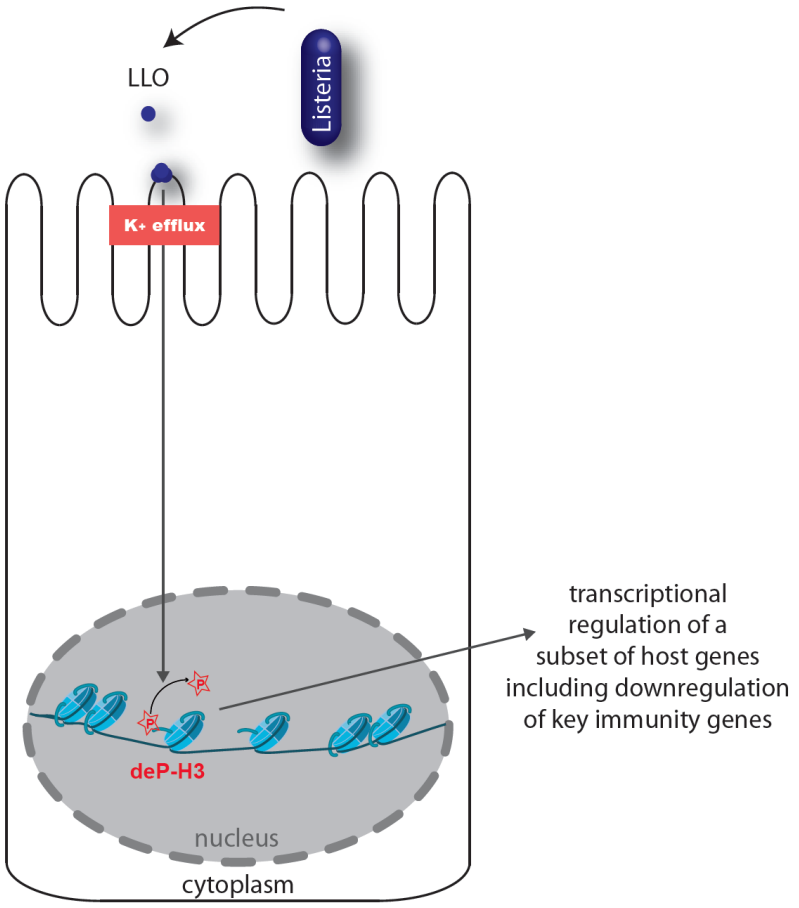
M. A. Hamon, P. Cossart, *Cell Host Microbe* **4**, 100 (2008).

L. Arbibe, *Cell Microbiol.* **10**, 1582 (2008)

Bacterial “nucleomodulins” target chromatin



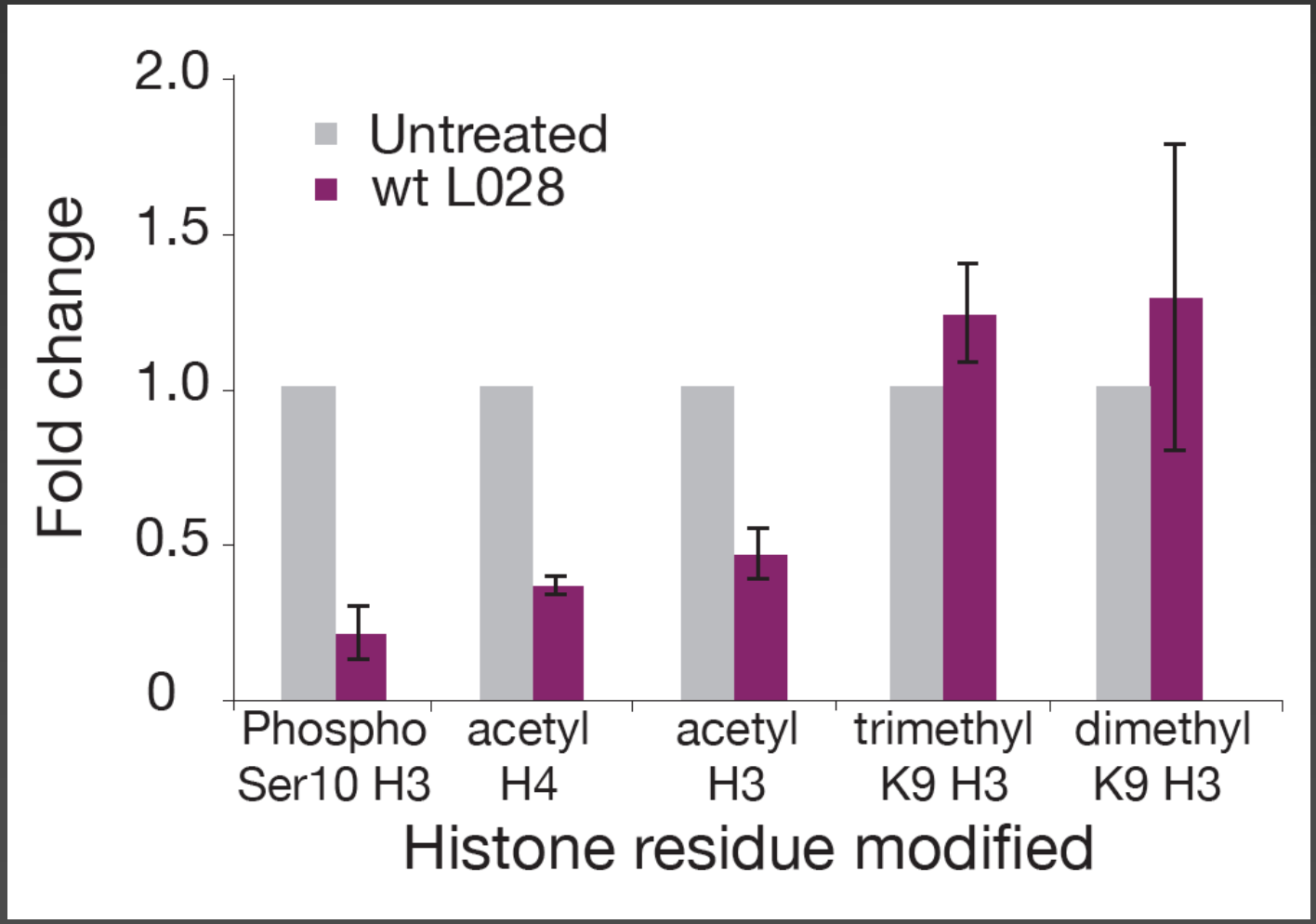
Dephosphorylation of histone H3S10 by a family of bacterial toxins



Hamon et al., PNAS, 2007
Hamon et al., IAI, 2011

- *L. monocytogenes* induces rapid and significant decrease in phospho H3 levels
- Listerolysin O (LLO) is the major factor inducing H3 dephosphorylation from the outside of the cell
- Potassium efflux through LLO pores is a signal leading to H3 dephosphorylation
- Decreased levels of phosphoH3 correlate with a reduced transcriptional activity of a subset of host genes
- Similar H3 dephosphorylation is observed with toxins of the same family as LLO (i.e. PLY, PFO)

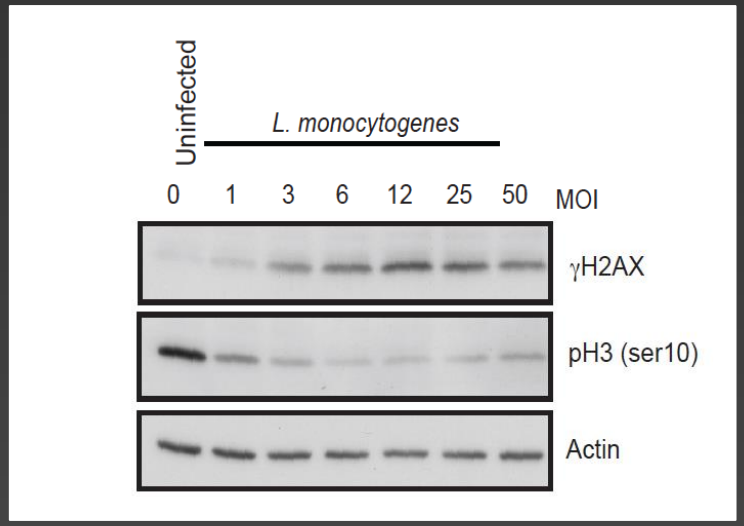
L. monocytogenes induces specific histone modifications during infection



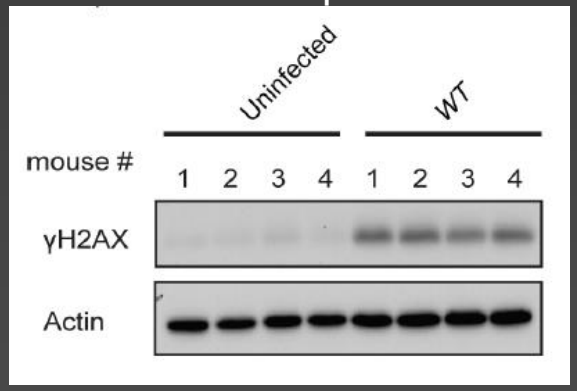
Hamon et al., PNAS, 2007

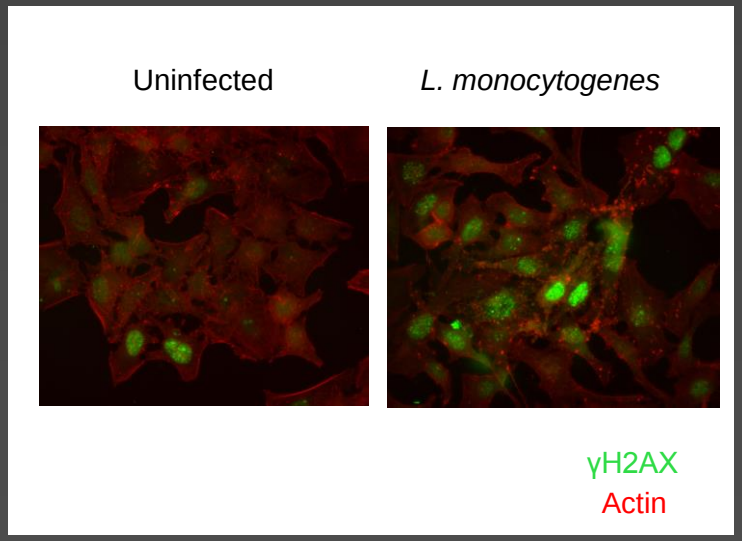
Listeria monocytogenes induces phosphorylation of H2AX (γH2AX)

Tissue culture

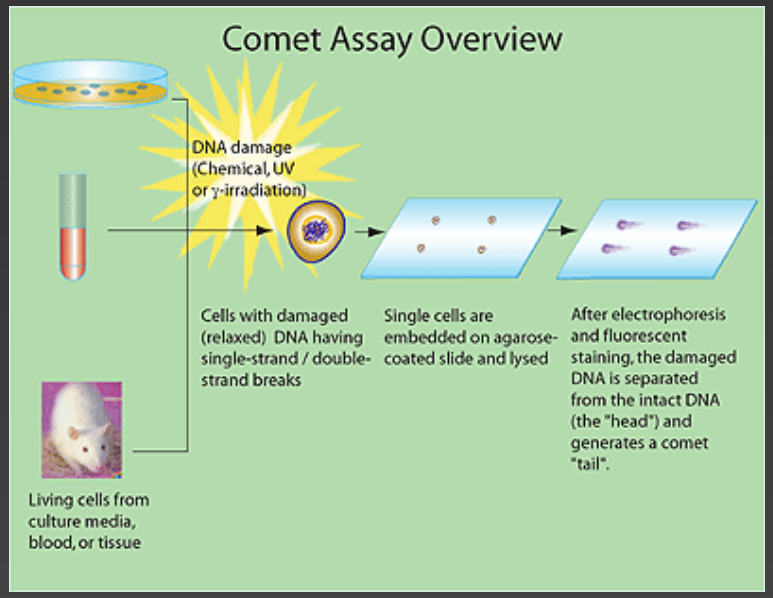


Mouse spleen

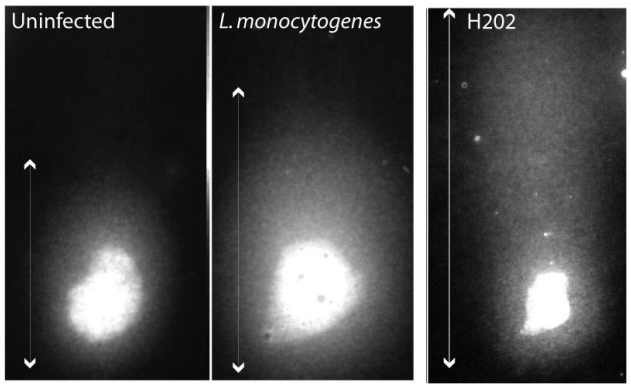


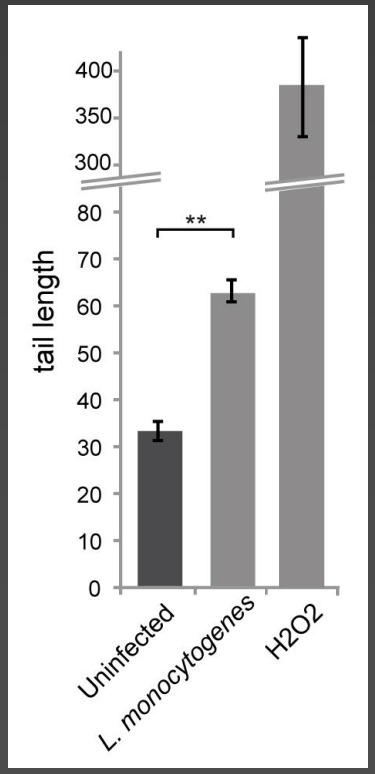


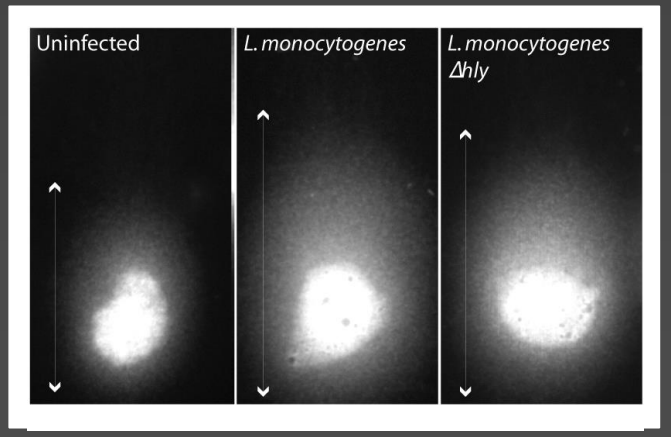
Listeria monocytogenes induces DNA breaks during infection



HeLa cells, 24h infection:

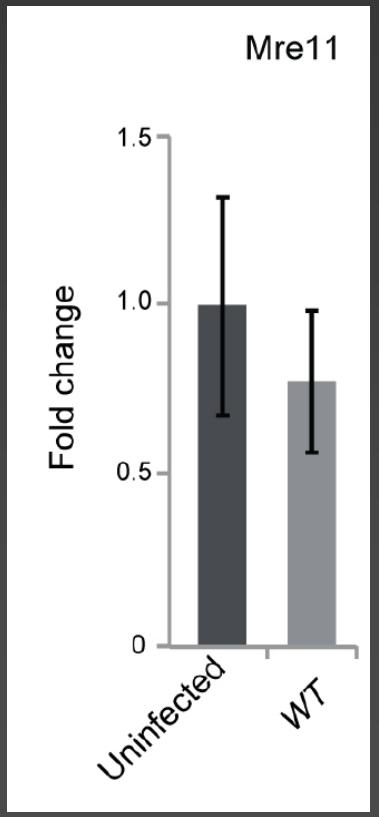




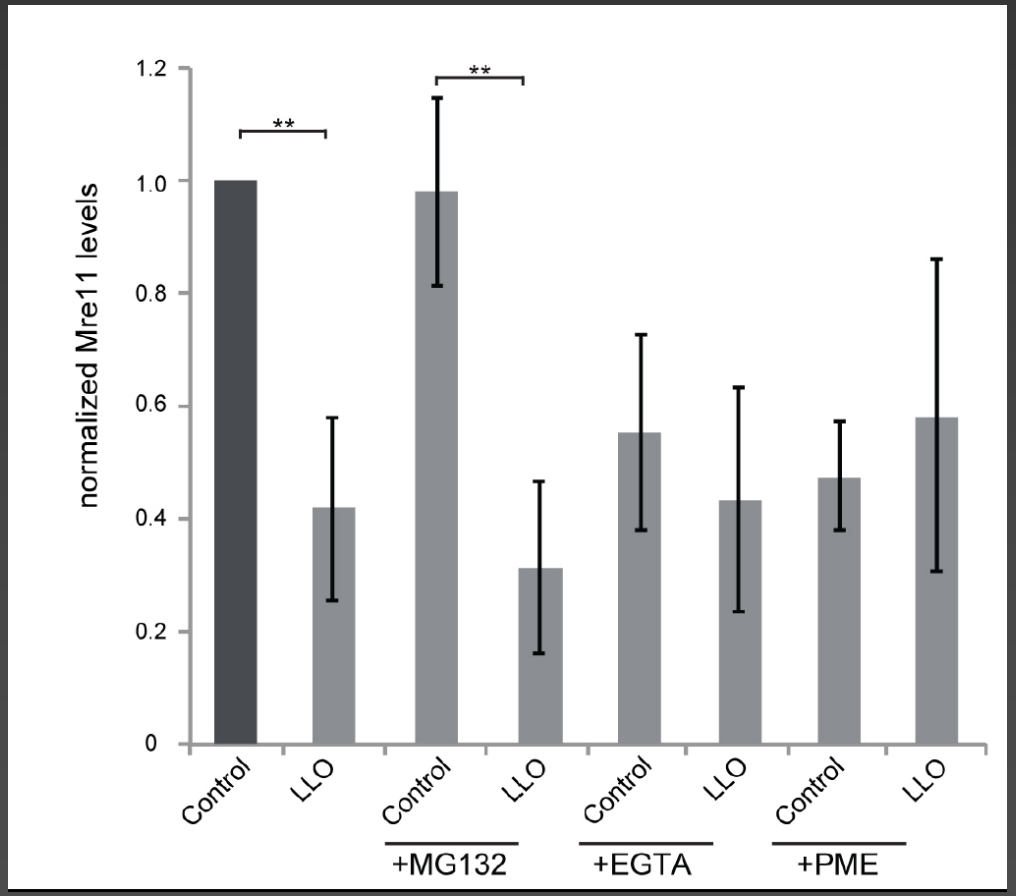


Mre11 is degraded by an aspartyl protease

Transcriptional level



Protein level

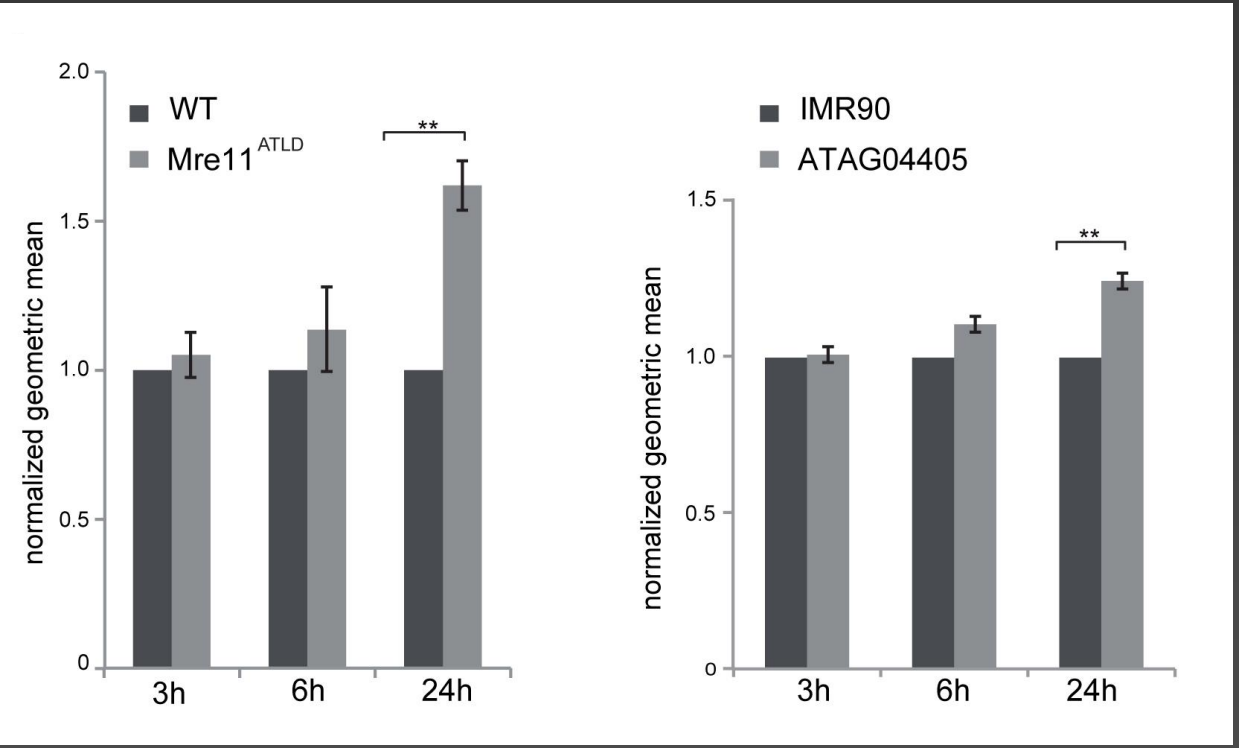


Proteasome inhibitor

Aspartyl protease inhibitor

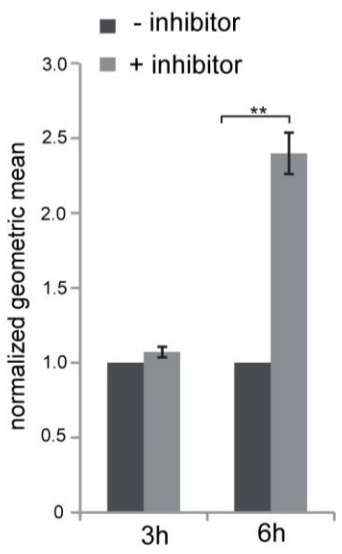
Dampening of the DDR is required for a productive infection

Cells infected with *L. monocytogenes* GFP and infection is monitored by FACS

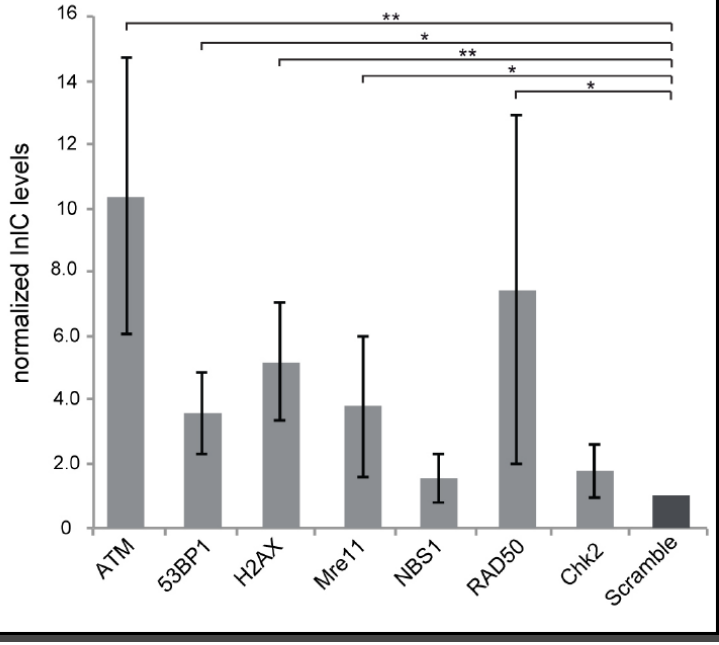


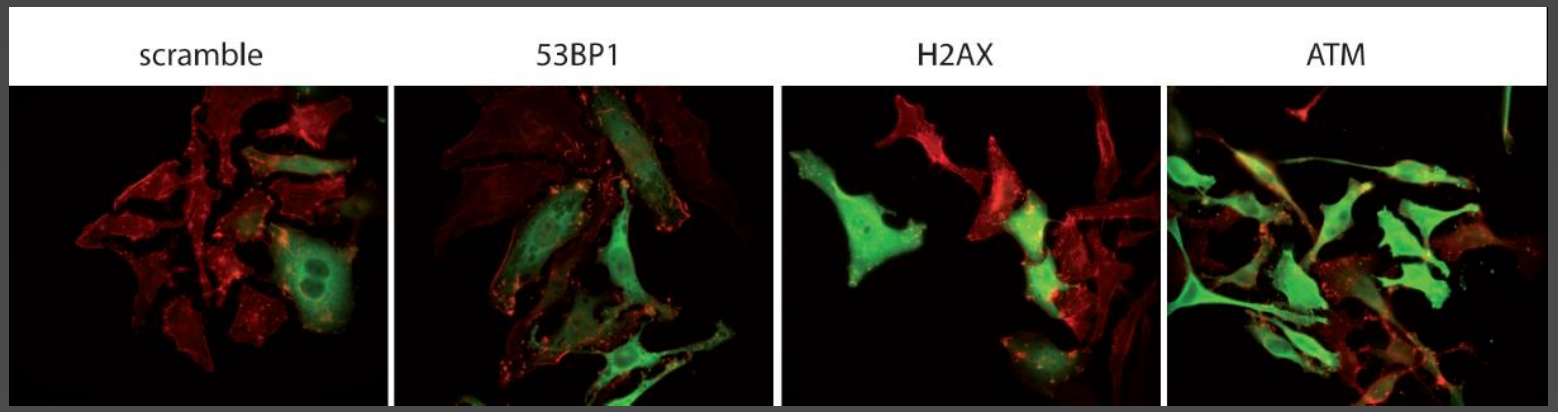
Dampening of the DDR is required for a productive infection

ATM inhibitor, KU60019



siRNA





InlC
Phalloidin

LLO induces a decrease in the levels of Mre11